Oxygen Club of California

University of Turin

OXIDANTS AND ANTIOXIDANTS IN BIOLOGY

BOOK OF ABSTRACTS

7-10 SEPTEMBER 2005

ALBA, ITALY

OXIDANTS AND ANTIOXIDANTS IN BIOLOGY

A MEETING IN HONOR OF ANGELO AZZI

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A JOINT MEETING OF
OXYGEN CLUB OF CALIFORNIA
UNIVERSITY OF TURIN

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KEYNOTE ADDRESS

Vitamin E
A paradigm of the mechanisms of scientific progress

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Scientific progress proceeds by a parallel development of techniques and novel ideas. Mitochondria were studied first as a "soluble enzyme" when the Michaelis-Menten mechanism for enzyme catalysis was dominating the biological field. It took biophysics, with the development of membrane concepts, to realize the possibility that chemiosmotic fluxes may be of relevance to the basic principle of energy conservation. The recent developments of signal transduction cascades have indicated that mitochondria are inserted in the signaling mechanism of a cell. Similarly tocopherols, early described as a vitamin that brings birth (animal studies) have been identified as redox compounds, capable of radical scavenging in a lipid phase, and thus protecting membrane from damage. This hypothesis (physicochemical studies) has attracted large sympathies due to its simplicity, but failed to explain differences among tocopherols having similar antioxidant properties and different cellular effects. The development of the concepts of cell signaling has given new impulses to the understanding of the molecular aspects of tocopherols action (cellular studies). Tocopherols have been assigned to the group of signaling molecules, rather than to that of antioxidants. The exclusive molecular effects of α-tocopherol indicate that its action is based on specific ligand binding properties. Cell regulation via protein kinase C and phosphatidyl inositol 3-kinase have been described that are possibly (but not exclusively) linked to gene transcription regulation. The recent finding that α-tocopheryl phosphate is present in cells in small amounts, that it can be synthesized and hydrolyzed supports the hypothesis that α-tocopheryl phosphate might be a signaling molecule and that the effects of α-tocopherol are exerted through its phosphorylation. The possible pathways needed for the synthesis, hydrolysis and signaling are considered as well the possible extension of this reaction to additional molecules, such as other tocopherols and tocotrienols.

SESSION I
OXIDANTS IN VASCULAR BIOLOGY

Lipid oxidation products disturb cholesterol homeostasis in vascular cells

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Cholesterol is an essential component of membranes, but in excess can cause cell dysfunction. In the vascular system, this includes altered endothelium-dependent reactivity and the formation of intimal macrophage-derived foam cells, both early markers of atherosclerosis. Homoestatic mechanisms normally limit excess cholesterol accumulation and promote cholesterol export, to maintain cell cholesterol within safe limits. Lipid oxidation products can alter the balance of these mechanisms in both beneficial and damaging ways. Expression of many of the lipid transporters and enzymes controlling cellular cholesterol are subject to oxysterol-dependent transcriptional regulation, mediated through oxysterol ligand-dependent transcription factors (LXR). Their ligands, such as 24(S),25-epoxycholesterol, are generated endogenously and at low concentrations by specific enzymes. In addition, several products of non-enzymic lipid oxidation can be detected in arterial tissue, generally at higher levels in atherosclerotic than in healthy vessels. The substrates appear to be mainly lipoprotein- derived fatty acids and cholesterol, which have become oxidized locally, either before or after uptake by intimal cells. Lipid oxidation products can promote cholesterol accululation directly and indirectly. Oxidation of esterified fatty acids can prevent their lysosomal hydrolysis, leading to lysosomal accumulation of undigested lipids. A major product of non-enzymic oxidation of cholesterol is 7-ketocholesterol (7KC). We have found that 7KC partitions selectively into lipid raft domains of the plasma membrane, where it disrupts the normal export of cholesterol from macrophage foam cells.

Oxidized cholesterol and atherosclerosis progression

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Oxysterols, namely 27 carbon atoms oxidation products of cholesterol, are found in relatively high amount in LDL from hypercholesterolemic individuals and consistently detectable in foam cells and necrotic core of human atherosclerotic lesion. At least in relation to inflammation and fibrosis, oxysterols appear to be strong activating molecules, contrary to the parental compound, provided with scarce or null effect per se. Of note, the challenge of cells of the macrophage lineage with a mixture of oxysterols like that detectable in hypercholesterolemic individuals leads to a marked overexpression of TGFβ1 but also MCP-1, CD36 and β1-integrin. The possible up-regulation of the last three proteins within the atherosclerotic lesion indicates the ability of biologically relevant amounts of oxysterols to attract monocytic cells into the subintimal space and to stimulate their differentiation into macrophages with eventual formation of adherent foam cells. Pro-inflammatory signalling triggered by oxysterols in macrophages primarily involves ERK pathway as confirmed by its marked prevention through the use of selective ERK inhibitors.

Heme oxygenase-1 mediates vasculo-protective effects of peroxisome proliferator activated receptors

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Ligand-induced activation of peroxisome proliferator activated receptors (PPARs) inhibits vascular inflammation, atherosclerosis and restenosis. These beneficial effects have been attributed to direct vasculo-protective and local anti-inflammatory properties of these nuclear receptors in cells of the vascular wall. We show that PPAR agonists induce expression of heme-oxygenase-1 (HO-1) in vitro as well as in murine carotid arteries. Furthermore we demonstrate a differential regulation of HO-1 expression by ligands for PPARγ and PPARα in endothelial cells (ECs), vascular smooth muscle cells V(SMCs) and macrophages. Inhibition of HO-1 enzymatic activity by zinc protoporphyrin and transfection with HO-1 siRNA, respectively, revealed a cell type-specific modulation of the inflammatory response by PPAR ligand-induced HO-1, which inhibited expression of COX-2 in VSMC, but not in EC. In contrast, in EC, HO-1 mediated the induction of cyto-protective genes such as PGD-synthase and VEGF by PPAR-ligands. Analysis of the human HO-1 promoter using transient-transfection and electrophoretic mobility shift assays confirmed that HO-1 is a direct PPAR target gene, whose transcription is regulated by two adjacent PPAR-responsive elements. Furthermore we demonstrate that a common polymorphism within the human HO-1 promoter critically influences its transcriptional regulation by PPARs. The discovery of HO-1 as a novel PPAR target gene will lead to a better understanding of the beneficial effects exerted by PPARs on the vascular wall and provides an additional rationale for the use of PPAR-activating drugs in the treatment of atherosclerosis and other inflammatory diseases.

Nrf2-mediated induction of antioxidant stress proteins in vascular cells

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Excessive generation of reactive oxygen species (ROS) in pre-eclampsia, atherosclerosis, diabetes and uraemia leads to an upregulation of antioxidant stress proteins in circulating blood, endothelial and smooth muscle cells. The transcription factor Nrf2 binds to the antioxidant responsive element (ARE) in the promoter region of target genes, e.g. HO-1, peroxiredoxin I. Keap1 sequesters Nrf2 in the cytoplasm in association with the actin cytoskeleton, facilitating proteosomal degradation of Nrf2. Oxidation of cysteine residues on Keap1 leads to Nrf2 translocation from the cytoplasm to the nucleus. Select kinases such as PKC, TGFβ-activated kinase and mitogen-activated protein kinases (MAPKs) modulate actions of Nrf2. We reported that 4-hydroxynonenal (HNE) induces expression of HO-1 and peroxiredoxin 1 (Prx 1) in human and murine vascular endothelial and smooth muscle cells. Increased HO-1 expression in atherosclerotic lesions may compensate for diminished bioavailability of NO and ameliorate ROS induced injury. Nrf2 translocation and HO-1 induction by HNE is significantly diminished in aortic smooth muscle cells derived from Nrf2-deficient mice (Ishii et al., Circ. Res. 94:609-616, 2004), rendering these cells more susceptible to oxidative injury. Homocysteine and HNE induced Prx 1 and HO-1 expression in fetal vascular smooth muscle isolated from normal pregnancies, whereas pre-eclamptic cells exhibited impaired antioxidant gene expression and abnormalities in MAPK activation. Our findings suggest that under conditions of sustained oxidative stress transactivation of ARE responsive genes may be compromised in pre-eclampsia.

Supported by Wellcome Trust, BBSRC, BHF, Japan Society for Promotion of Science.

Effects of flavonoids on vascular function

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Epidemiological studies suggest that increased flavonoid intake reduces cardiovascular risk. Endothelial dysfunction is associated with a vasoconstrictor, prothrombotic, and proinflammatory state that may contribute to the pathogenesis of cardiovascular disease. Prospective studies have shown that endothelial dysfunction is associated with an increased cardiovascular risk. Many interventions known to reduce cardiovascular disease risk have the ability to reverse endothelial dysfunction. These findings suggest that endothelial function may have utility as a surrogate marker of cardiovascular risk. Recent studies suggest that flavonoid-containing foods improve the function of the endothelium. For example, several studies have shown that tea consumption improves endothelial function. Treatment with the tea catechin EGCG also improves endothelial function. Although tea-derived flavonoids have antioxidant properties, there currently is little evidence that an antioxidant effect explains the observed benefit of tea. In this regard, tea consumption does not alter plasma antioxidant capacity, plasma F2 isoprostanes, or 8-hydroxydeoxyguanosine. However, recent studies suggest that tea flavonoids have direct effects on the endothelial isoform of nitric oxide synthase and increase production of nitric oxide in endothelial cells. Endothelial function is also improved following consumption of other flavonoid-containing foods, including grape juice, de-alcoholized red wine, and chocolate. In summary, epidemiological and mechanistic studies suggest that flavonoids have beneficial effects on the cardiovascular system and may reduce the risk of cardiovascular disease. One potential mechanism for reduced cardiovascular risk is improved endothelial function. Confirmation of the clinical benefit of flavonoids for the primary or secondary prevention of cardiovascular disease will require randomized clinical trials.

Regulation of 5'-AMP-activated kinase, an energy-sensing enzyme, in endothelial cells

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The 5'-AMP-activated kinase (AMPK) plays a key role in regulating cellular energy metabolism. It is activated by a rise in the AMP:ATP ratio in times of reduced energy availability and serves to reduce ATP-consuming and to support ATP-generating pathways. In endothelial cells AMPK has been shown to phosphorylate endothelial NO synthase (eNOS) at serine 1177 and to contribute to eNOS activation. The present study investigates the regulation of AMPK in endothelial cells from human umbilical cord veins. We show that AMPK is not only activated by energy depletion or by 5-amino-4-imidazole carboxamide riboside which mimics the activatory effects of AMP, but also via receptor-dependent pathways and upon oxidative stress. AMPK activation involves its phosphorylation and was measured with phosphospecific antibodies against AMPK and its substrate acetyl-CoA carboxylase. Our data demonstrate that thrombin stimulation of endothelial cells activates AMPK without changing intracellular ATP via a signaling pathway involving protease-activated receptor-1, Gq-protein, phospholipase C and calcium. In parallel, eNOS was phosphorylated at serine 1177, which was most probably related to AMPK activation. Treatment of cells with hydrogen peroxide also leads to a time- and dose-dependent AMPK activation. Under conditions of oxidative stress, however, eNOS is uncoupled and AMPK-induced eNOS phosphorylation may not support the generation of NO. Our data suggest that activation of AMPK couples cell signaling pathways and oxidative stress to the regulation of metabolic enzymes and eNOS in physiological and pathophysiological conditions.

Nitric oxide and nitric oxide-derived oxidants: Interactions with human LDL

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Nitric oxide ($^{\bullet}NO$) reacts fast with lipid radicals and may exert antioxidant actions in the intravascular space by termination reactions during LDL oxidation. Using fluorescence quenching of pyrene derivatives, we established that $^{\bullet}NO$ can readily diffuse to the surface and core of LDL ($D'_{NO} \sim 2 \times 10^5$ $cm^{-2}s^{-1}$) and by this property serve as a major lipophilic antioxidant in LDL. Moreover, direct measurements of the partitioning of $^{\bullet}NO$ into liposomes and LDL showed a 3~4-fold increase in its concentration in hydrophobic compartments in comparison to the surrounding aqueous milieu. With this new data, the actual D_{NO} value was re-calculated as $\sim 4 \times 10^6$ $cm^{-2}s^{-1}$, about 10-times smaller than in water. The product of the partition ($K_{P(h/w)}$) and diffusion coefficients is a good indicator that mechanistically supports the diffusion-controlled termination reactions between $^{\bullet}NO$ and lipid radicals that effectively occur in lipid phases. On the other hand, peroxynitrite, the product of the $^{\bullet}NO$ and superoxide reaction, directly promotes LDL lipid and protein oxidation indicating that a redox imbalance in the vasculature can readily shift the anti-oxidant actions of $^{\bullet}NO$ towards a pro-oxidant reactivity. Both, $^{\bullet}NO$ and peroxynitrite reactions during lipid oxidation lead to the formation of nitrated lipids, which have been recently detected both *in vitro* and *in vivo*. Most interestingly, nitrated lipids are being unraveled as strong cell signaling mediators that can play potent anti-inflammatory actions.

SESSION II
DIETARY AND METABOLIC OXIDATIVE STRESS

Postprandial glucose regulation and diabetes implications

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There is increasing evidence that the postprandial state is an important contributing factor to the development of atherosclerosis. In diabetes, the postprandial phase is characterized by a rapid and large increase in blood glucose levels, and the possibility that the post-prandial "hyperglycemic spikes" may be relevant to the pathophysiology of late diabetic complications has received recently much more attention.The oral glucose tolerance test, although higly non-physiological, has been used largely as model of the post-prandial state. Epidemiological studies have shown that, when impaired, oral glucose tolerance is associated with an increased risk of cardiovascular disease, being the glycemia after two hours of the glucose challenge a direct and independent risk factor. Moreover, the possibility that post-prandial hyperglycemia is a risk factor for cardiovascular disease also in diabetic patients has been reported.Most of the cardiovascular risk factors are modified in the post-prandial phase in diabetic subjects and directly affected by an acute increase of glycemia.The mechanisms through which acute hyperglycemia exerts its effects may be identified in labile non-enzymatic glycation and in production of free radicals. It is likely that the two mechanisms co-operate in causing the disorders induced by acute hyperglycemia. Correcting the postprandial hyperglycemia can form part of the strategy for the prevention and management of cardiovascular diseases in diabetes.

A new perspective on obesity: Early metabolic malprogramming

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The staggering increase in the incidence of obesity, type 2 diabetes and related metabolic disorders world-wide has prompted intense research into the etiology of these disorders. In this context, the role of an early life dietary modification (high carbohydrate; HC) during the suckling period in the rat is being investigated in our laboratory. This nutritional modification results in the immediate onset of hyperinsulinemia, its persistence into adulthood and adult-onset obesity (HC phenotype) in first generation (1-HC) rats. Adaptations at the biochemical, cellular and molecular levels in the islets of HC rats support the early onset of hyperinsulinemia. The hypothalamus also responds to this dietary modification via increases in the immunoreactivity of neuropeptide Y and galanin and a decrease in the expression of the insulin receptor α subunit. These early metabolic responses continue to be expressed in adulthood. 1-HC females spontaneously transmit the HC phenotype to their progeny (second generation HC rats; 2-HC). In the absence of maternal hyperglycemia, hyperinsulinemia is observed in 2-HC fetuses as early as on fetal day 20. In the hypothalamus increases in the immunoreactivity for neuropeptide Y and galanin and a decrease in the immunoreactivity of the insulin receptor α submit are observed on fetal day 20. Our results indicate the vulnerability of the immediate postnatal period for long-term effects on metabolic malprogramming due to calorie redistribution rather than a change in the total calorie availability as observed in conventional nutritional rodent models.

Macronutrient intake induces oxidative and inflammatory stress while insulin causes suppression of ROS generation and inflammation

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Following our original observation that the intake of 75g of glucose in normal subjects induces an increase in ROS generation by mononuclear cells (MNC), we have shown that glucose, equicaloric amounts of fat (eaten as cream) and a mixed fast food meal (900 calories) induce not only an increase in ROS generation by MNC but also cause an increase in p47 phox expression. In addition, there is an increase in intranuclear NFκB binding, a fall in IκBα expression and an increase in IKKα and IKKβ expression. There is a concomitant increase in TNFα mRNA in the MNC. Two other pro-inflammatory transcription factors, activator protein-1 (AP-1) and early growth response-1 (Egr-1), were also induced by glucose intake. There was an increase in MMP-2, MMP-9, tissue factor (TF) and PAI-1. Thus, there occurs a comprehensive oxidative and inflammatory stress response following macronutrient intake. Consistent with this concept, the state of obesity, associated with increased macronutrient intake, is characterized by an increase in oxidative stress and chronic low grade inflammation. As would be expected, caloric restriction in the obese results in a marked reduction in ROS generation by MNC and other indices of oxidative stress, like lipid peroxidation and protein carbonylation. A 48 hour fast in normal subjects leads to a reduction in ROS generation by 50% and a parallel reduction in p47phox. In contrast to macronutrient intake, a low dose insulin infusion (2 units per hour), results in a significant reduction in ROS generation by MNC, p47phox expression, intranuclear NFκB binding with an increase in IκBα expression. In addition, there is a suppression of AP-1 and Egr-1, MMP-2, MMP-9, PAI-1 and tissue factor (TF). This allows us to conclude that there exists a novel relationship between macronutrient intake and insulin, the hormone secreted in response to macronutrient intake. This extends beyond the classical relationship involving metabolic mechanisms only. We are now engaged in the investigation of foods which are least likely cause oxidative and inflammatory stress. Alcohol, orange juice and a high fiber and fruit containing meal do not cause oxidative of inflammatory stress.

IKKβ links inflammation to obesity-induced insulin resistance

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Inflammation may underlie the metabolic disorders of insulin resistance and type 2 diabetes. IκB kinase β (IKKβ) is a central coordinator of inflammatory responses through activation of NF-κB. To understand the role of IKKβ in insulin resistance, we used mice lacking this enzyme in hepatocytes ($Ikk\beta^{\Delta hep}$) or myeloid cells ($Ikk\beta^{\Delta mye}$). $Ikk\beta^{\Delta hep}$ mice retain insulin responsiveness, but develop insulin resistance in muscle and fat in response to high fat diet, obesity or aging. In contrast, $Ikk\beta^{\Delta mye}$ mice retain global insulin sensitivity and are protected from insulin resistance. Thus, IKKβ acts locally in liver and systemically in myeloid cells, where NF-κB activation induces inflammatory mediators that cause insulin resistance. These findings demonstrate the importance of liver cell IKKβ in hepatic insulin resistance and the central role of myeloid cells in development of systemic insulin resistance. We suggest that inhibition of IKKβ, especially in myeloid cells, may be used to treat insulin resistance.

Oxidative stress acts as trigger of autoimmune reactions in alcoholic liver disease

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Growing evidence indicates that inflammatory and immune reactions are involved in the pathogenesis of alcoholic liver disease (ALD). The contribution of oxidative stress to the immune responses associated with ALD has emerged form the detection of antibodies directed against protein adducts with and several lipid peroxidation products in patients with advanced ALD. These patients also display T-lymphocyte response toward lipid peroxidation antigens, indicating the involvement of oxidative damage in promoting both humoral and cellular immune reactions. Furthermore, we have observed that the alkylation of cytochrome P4502E1 (CYP2E1) by hydroxyethyl free radicals (HER) generated during CYP2E1-mediade ethanol metabolism not only stimulates the formation of specific anti-HER antibodies, but also favours the development of anti-CYP2E1 auto-antibodies. The breaking of self tolerance during ALD is greatly enhanced by an impaired control of T lymphocyte proliferation due to genetic polymorphisms of immuno-regulatory molecule CTLA-4. High titres of anti-CYP2E1 auto-antibodies are detectable in about 40% with advanced ALD and their presence correlates with the extension of lymphocyte infiltration and the number of apoptotic hepatocytes. Such effect is likely due to the capacity of anti-CYP2E1 auto-antibodies to trigger antibody-dependent cell-mediated cytotoxicity by targeting CYP2E1 present on the outer layer of hepatocyte plasma membranes. Altogether, these observations indicate the importance of ethanol-induced oxidative stress in stimulating both allo- and auto-immune reactions and suggest a possible role of immunological mechanisms in the progression of hepatic injury by alcohol.

SESSION III
ENVIRONMENTAL OXIDATIVE STRESS

Solar ultraviolet A radiation-induced biological effects in human skin

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Solar ultraviolet radiation-induced biological effects in human skin. Ultraviolet A radiation (UVAR) exerts dementrial effects on human skin. It is thus imporant to study the molecular mechanisms underlying these effects. In previous years we have shown that exposure of primary human epidermal keratinocytes (NKH) to UVAR at physiological doses leads to increased gene expression such as ICAM-1, which is mediated through activation of transcription factor AP-2 [1]. Subsequently we have identified a non-enzymatic triggering of the ceramide signalling cascade as the initiating step in UVA radiation-induced signaling [2]. These signaling ceramides act on mitochondria and induce the release of non-apoptogenic amounts of cytochrome C, which through a redox regulation causes activation of AP-2 [3]. Next, we have shown that the UVAR-induced generation of ceramide occurs at the level of the cell membrane sphingomyelin through a process that involves the generation of singlet oxygen. Within the plasma membrane, sphingomyelin is not equally distributed, but preferentially present in microdomains, also called "rafts", which additionally contain high amounts of cholesterol. Our studies indicate that UVAR-induced signaling involves cell membrane lipid rafts. This assumption is based on the observation that (i) alteration of the cholesterol content of cell membrane lipid rafts dramatically affects the capacity of NHK to mount a UVA response and (ii) that gene knock-down of the raft-associated protein caveolin-1 by means of siRNA almost completely inhibits UVA radiation-induced gene expression. We have therefore started to analyze the mechanisms through witch UVAR activites Caveolin-1. Our most recent results indicate in a critical role for src kinase yes and fin in this context. The identification of cell membran lipid rafts and raft-associated proteins as integral companients of the UVAR-induced stress response provide the basis for the development of novel strategies to protect human skin against UVAR-induced detrenital effects.

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Antioxidant micronutrients in skin health

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As other tissues, skin depends on an optimal supply with nutritive compounds. Skin benefits from dietary antioxidants capable of scavenging reactive intermediates generated under the condition of photooxidative stress. Micronutrients may also act as UV absorbers, or modulate signaling pathways elicited upon UV exposure (1). Based on their structural features which determine the physico-chemical properties, carotenoids, flavonoids, as well as vitamin E and C are suitable compounds for photoprotection in humans. Intervention studies with carotenoid supplements or diets rich in carotenoids have shown that these compounds contribute to systemic photoprotection ameliorating UV-induced erythema (2,3). Protection through individual dietary components in terms of sun protection factor (SPF) is considerably lower than that achieved using topical sunscreens, however, an increased life-long overall protection via dietary supply may contribute significantly to skin health. Little is known about the effects of carotenoids, vitamins and trace elements on skin texture, structure and microcirculation, parameters which are closely related to skin health and aging. Premature aging of the skin results in increased wrinkling and a loss of elasticity. Supplementation with dietary micronutrients modulates skin structure and texture contributing to the resistance of skin to environmental stress and improving general parameters indicative for skin health.

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Oxidant-induced signaling events in asbestos-related diseases

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Asbestos is a naturally occurring oxidant stress after inhalation and causes lung carcinomas, mesotheliomas and pulmonary/pleural fibrosis. We have shown that PKCδ is increased in mouse lungs after inhalation of asbestos and is critical to the mitochondrial death pathway after exposure of lung epithelial cells to asbestos. Here we used PKCδ knockout mice (PKCδ-/-) to address a possible role for PKCδ in lung inflammation and fibrosis using a murine asbestos inhalation model. Exposure to asbestos for 9 days resulted in elevation of total cell counts within bronchoalveolar lavage fluid (BALF) that was comparable between wild type (wt) and PKCδ-/- mice. Differential cell counts revealed a predominance of neutrophils (~80%) in BALF from asbestos-exposed wt mice, whereas increased numbers of neutrophils (~30%), CD4+ and CD8+ lymphocytes (~20%), and alveolar macrophages (~30%) were observed in asbestos-exposed PKCδ-/- mice. Cytokine profiles (TNFα, IFNβ, IL-1β and IL-6) were measured in BALF by ELISA following 9 and 40 day asbestos exposures. Comparable increases in IL-1β and IL-6 were observed in sham and asbestos-exposed wt and PKCδ-/- mice at 9 days, whereas TNFα levels did not change. IL-6, but not IL-1β remained elevated at 40 days in asbestos-exposed wt and PKCδ-/- animals. In contrast, IFNβ levels were lower in sham and asbestos-exposed wt mice at 9 days. Asbestos-induced lung fibroblast proliferation and pulmonary fibrosis were inhibited in PKCδ-/- mice, suggesting a role of PKCδ in inhibition of cell proliferation. We are presently addressing the mechanisms of this response in isolated fibroblasts from normal and PKCδ knockout mice.

Research was supported by P01 HL67004 from NHLBI.

Noninvasive laser detection of carotenoid antioxidants in human tissue

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Motivated by the growing importance of carotenoids in health and disease, we developed Raman and fluorescence spectroscopy for the noninvasive rapid optical detection of carotenoids in living human tissue. In the human retina, the macular carotenoid pigments lutein and zeaxanthin are thought to play a protective role via filtering and/or antioxidant function, and thus may prevent or delay the onset of age-related macular degeneration. We developed several ocular instruments to measure both integral and spatially resolved carotenoid distributions. In large populations of normal subjects we find a decline of average macular carotenoid levels with age. In populations with macular pathologies we find reduced pigment levels as compared to age-matched controls. In the human skin, about half a dozen carotenoids are present. Using portable Resonance Raman instruments we measured skin carotenoid levels of large populations, and obtained evidence that optically derived skin carotenoid readings are useful as a surrogate marker for general antioxidant status. The measurements showed that subjects with high oxidative stress, like smokers, and subjects with high sunlight exposure, have in general reduced skin carotenoid levels, independent of their dietary carotenoid consumption. We find the Raman and fluorescence techniques to be precise, specific, sensitive, and well suitable for clinical as well as field studies. The non-invasive laser techniques may become a useful method for the correlation between tissue carotenoid levels and risk for malignancies or other degenerative diseases associated with oxidative stress.

Carotenoid diets modify mouse lung responses to xenobiotic metabolism, oxidative stress, and inflammatory-immune processes

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The mechanism(s) of tissue interaction between carotenoid (CAR) intake and inhaled cigarette smoke (CS) remain to be clarified. Affymetrix Mouse 430A 2.0 arrays were used to determine the mRNA expression changes in C57BL6 mouse lung in response to CS (60 mg particulates/m^3, 6h/day for 3 days) and CAR dietary supplements. The total number of lung genes affected by either CAR or CS is <0.1% or ~1.0% respectively of the ~15,000 genes detected. Dietary supplementation with CAR (β-carotene, lycopene, or lutein or an equal mixture of the 3 CAR from BASF) modulated the effect of CS on lung gene expression. Diets with an equal mixture of the 3 CARs with CS exposure modified more genes (1.44%) than individual CAR. In CS-exposed mice CAR affects lung expression of transcripts related to xenobiotic metabolism, oxidative stress and inflammatory-immune res- ponses. A companion human study investigated the effects of carotenoids on DNA damage in humans. Gene array (Affymetrix HU-133) analysis was performed on RNA isolated from whole blood. A high abundance of globin genes and many clusters of genes encoding cytokines and cell surface markers associated with white blood cells were identified, but due to large variations reliable analysis of these data could not be made. Yeum *et al*. using the comet assay demonstrated that a physiologic dose of the CAR mixture or a larger dose of the individual carotenoids protects lymphocytes against endogenous or H_2O_2-induced DNA damage.

Quantitative changes in TTP, SRB1 and ABCA1 gene expression in murine lung tissue upon exposure to combined environmental pollutants. Differences between old and young

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Despite the physiological importance of vitamin E, in particular the α-tocopherol isoform, the molecular mechanism involved in the cellular uptake of this antioxidant from the plasma lipoproteins have not been well defined. Plasma α-tocopherol concentrations are regulated by hepatic α-tocopherol transfer protein (TTP). α-Tocopherol is mainly transported in HDL lipoproteins. Recent studies have suggested that selective lipid uptake is important for α-tocopherol delivery to cells. It has been shown that SRB1 facilitates α-tocopherol transfer from HDL to the cells. Furthermore ABCA1 mediates secretion of cellular α-tocopherol into the HDL metabolic pathway, facilitating vitamin transport between tissues. In this study we want to asses whether aging and environmetal pollution can modulate this pathway. To explore the validity of the hypothesis, determined the effects of pollutants such as UV (0.3 MED), O_3 (0.25 ppm 6 hr per day) and ETS (60 mg/m^3 6 hr per day) alone or in combination for 4 days on young lung and aged lung using hairless mice as *in vivo* model. RNA was extracted from the whole lung tissues and real time PCR was used to quantify gene expression. Results showed that old animals resulted to have a significant decrease in TTP and SRB1 mRNA compared to the young. O_3 as well as ETS induced a further down-regulation of both genes. ABCA1 did not change in old compared to the young animals. These data suggest that aging as well as environmental pollution can affect vitamin E transport and uptake from and to the tissue.

Oxidative stress as a common pathogenic determinant in chronic degenerative diseases

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We evaluated the consequences of oxidative DNA-damage (ODD) in various experimental conditions by monitoring 8-hydroxy-2'-deoxyguanosine by ^{32}P-post-labelling and gene-expression by cDNA array. In mouse foetal liver, the low basal level of ODD was increased following transplacental exposure to cigarette smoke. The foetus counteracted ODD by increasing the expression of genes inhibiting cell replication and triggering apoptosis (FASEB J.,17:1127, 2003). Accordingly, smoke-induced ODD in foetus resulted in growth retardation. During the foetus-newborn transition, the acquisition of independent respiratory function triggered the expression of genes involved in the removal of oxidized proteins and detoxification of oxidative species in mouse lung (Mutat. Res. Rev., 544: 441, 2004). These alterations were attenuated by administering the antioxidant N-acetyl-L-cysteine to the dams during pregnancy. In humans, we found that ODD is consistently detectable in the aorta of atherosclerotic patients (FASEB J., 11: 1021, 1997), being 4-fold higher in the endothelium than in the medium layer. To substantiate the hypothesis that ODD is related to various chronic-degenerative diseases, we analyzed the level of ODD in patients affected by primary open angle glaucoma, the main cause of irreversible blindness worldwide. We found that ODD is significantly increased in the trabecular meshwork, the specialized epithelium regulating the intraocular pressure, of glaucoma patients as compared to unaffected controls (Am. J. Med, 114: 638, 2003). This situation leads to an increase of the intraocular pressure resulting in optic nerve alterations and visual field defects. Altogether, these data support the hypothesis that ODD is involved in a variety of physiological process (*e.g.*, birth) and pathological conditions (*e.g.*, cancer, atherosclerosis, glaucoma).

SESSION IV
REDOX REGULATION OF CELL SIGNALING AND GENE EXPRESSION

Electrophile-sensitive proteins

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Many conditions, including oxidative stress and inflammation, endogenously generate a variety of small chemicals. These chemicals include lipid peroxidation-derived α,β-unsaturated aldehydes and prostaglandin metabolites. Many of them are strong electrophiled and, therefore, show the highest reactivity with nucleophiles, such as proteins and DNA. Similar electrophiles are generated as ubiquitous pollutants in the environment and causally involved in the pathophysiological effects. Food materials, such as vegetables and fruits, also contain a variety of electrophiles, including isothiocyanates and phenolic compounds, some of which confer resistance to a broad set of extracellular stimuli. Thus, electrophiles are closely linked to personal health. To elucidate the molecular mechanism by which these endogenous and exogenous electrophiles induce their unique spectrum of biological effects, we undertook proteomics on electrophile sensitive proteins. We have identified some candidate molecules, which could be implicated in mediating the cellular responses induced by electrophiles. The causal relation between the targets of electrophiles and its biological effects will be discussed.

Redox control in the endoplasmic reticulum

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Many secretory proteins contain disulfide bonds that are essential for folding and function. The process of achieving the correct tertiary structure and tying it with disulfide bonds occurs in the ER, under the assistance of specialized protein machineries that control the conformity of the result and finally decide the fate of the newly formed protein (quality control). The main oxidative folding pathway requires the transfer of oxidative equivalents to newly synthesized cargo proteins via PDI, which in turn is oxidized by Ero1 proteins. Two members of the family exist in mammals, Ero1alpha and Ero1beta. The latter is induced during ER stress conditions, when the demand for oxidative power may increase. However, the process of ER quality control require extensive disulfide isomerization during folding and reduction from terminally misfolded proteins prior to their retrotranslocation to the cytosol for proteasomal degradation. These reactions require some PDI to be in the reduced state. Therefore, oxidation must be limited. The import of cytosolic GSH balances the oxidative power of Ero1. The mechanisms that regulate Ero1 activity also involve sequential interactions with PDI and ERp44, a novel folding assistant of the secretory pathway. Ero1alpha over-expression increases the GSH content in HeLa cells, implying the existence of intercompartmental regulatory pathways that control redox signalling and homeostasis.

Key words: Disulfide bonds; ER-associated degradation; Ero1; glutathione; oxidative folding; PDI; protein secretion; quality control; ER-associated degradation; Ero1 / oxidative folding; PDI / quality control.

Molecular mechanism of sulfiredoxin

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Sulfiredoxin (Srx) has been identified as an enzyme responsible for the reduction of cysteine sulfinic acid of peoxiredoxin (Prx) to cysteine in yeast and mammalian cells. A number of thiols like glutathion (GSH), thioredoxin, and ditihothreitol can provide reducing equivalents. Based on the requirement of ATP hydrolysis for the reduction and on the observation that Srx and Prx form a disulfide-linked complex during catalysis, yeast Srx was proposed to function as a phosphotransferase as well as a thioltransferase. Here we studied a molecular mechanism by which human Srx reduces cysteine sulfinic acid of Prx I to cysteine by following ATP hydrolysis catalyzed by Srx. Srx binds to ATP independently of Prx. Srx hydrolyzes ATP α-phosphate in the presence of sulfinic PrxI (Prx I-SO2) but not in the presence of reduced Prx I (Prx I-SH). The final level of released α-phosphate was equal to that of sulfinic Prx I in the presence of GSH. In the absence of GSH, however, the ATPase reaction proceeded much longer resulting in the amount of α-phosphate that is much larger that that of sulfinic Prx I. This implies that the sulfinic phosphoryl ester is directly attacked by the thiols such as GSH, thioredoxin and DTT rather than by the catalytic cysteine of Srx. From mutational analysis of conserved residues of rat Srx, it was identified that Arg^{50} and Asp^{79} are involved in interaction with Prx and Lys^{60}, Cys^{98}, His^{99} and Arg^{100} are required for phosphotransferase activity of Srx.

Redox control of the ASK1-MAP kinase signaling in apoptosis and immunity

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein (MAP) kinase kinase kinase family, which activates both the MKK4/MKK7-JNK and MKK3/MKK6-p38 MAP kinase pathways. ASK1-JNK/p38 cascade constitutes an important signaling pathway in various types of stress-induced apoptosis. We have shown by deletion of Ask1 gene in mice that ASK1 plays pivotal roles in oxidative stress- and endoplasmic reticulum (ER) stress-induced apoptosis. These stresses are closely linked to various physiological phenomena in the control of cell fate, and the resultant apoptosis is implicated in the pathophysiology of a broad range of human diseases. Moreover, ASK1-p38 pathway was recently found to play important roles in the innate immune responses. In this symposium, I will review our recent findings on the pathophysiological roles of ASK1 in stress responses.

An ASJ neuron-specific thioredoxin in *Caenorhabditis elegans*

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The thioredoxin system, composed of thioredoxin (Trx) and thioredoxin reductase (TrxR), has emerged as a key player in cellular redox-mediated reactions in both prokaryotic and eukaryotic cells. The *C. elegans* genome codes for 9 thioredoxins and 2 thioredoxin reductases, most of them being highly conserved through evolution. We performed expression analyses of the entire thioredoxin gene complement using promoter as well as translational fusions to GFP. Interestingly, we discovered unexpected expression patterns not previously found in any other organism, especially in the context of tissue-specificity. One of the Trx genes, B0228.5, was shown to be expressed only in the ciliated sensory neuron ASJ, in both neurites and cell body, in what is the first report of a thioredoxin expressed exclusively in neurons. The gene is expressed from L1 to adulthood in both males and hermaphrodites. The B0228.5 gene codes for a 115 amino acid protein (Trx-115) lacking any targeting sequence for specific subcelullar localization. When expressed in bacteria, recombinant Trx-115 is able to reduce protein disulfide bonds in the presence of TrxR and NADPH, confirming that Trx-115 is a functional thioredoxin. We also performed RNAi feeding studies and so far found no visible phenotypes. A more detailed screen for neuron-specific phenotypes is under way. The results emerging from work in *C. elegans* will have an important impact on mammalian studies. In particular, the identification of the first neuron-specific thioredoxin paves the way to establish an easy-to-work platform to study the role of thioredoxins in human nervous system diseases and aging.

Signaling of apoptosis by SecTRAPs Linking the integrity of selenocysteine in thioredoxin reductase with cellular effects triggered by electrophilic anticancer agents

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SecTRAPs (selenium compromised thioredoxin reductase- derived apoptotic proteins) are potent inducers of apoptosis, formed by derivatization of thioredoxin reductase (TrxR) with electrophilic agents or by removal of selenocysteine in TrxR by C-terminal truncation.1 TrxR is rapidly targeted by the major classes of clinically used electrophilic anticancer agents, while, in contrast, glutathione reductase is not. 2 Apoptosis triggered by SecTRAPs in A549 or HeLa cells did not require induction of protein synthesis. A pan-caspase inhibitor fully protected cells from SecTRAPs, illustrating caspase activation. Using more specific caspase-2 and -3 inhibitors suggested that activation of both of these caspases was a prerequisite for apoptosis induction by SecTRAPs. HeLa cells overexpressing Bcl-2 were as sensitive to SecTRAPs as parent HeLa cells, in sharp contrast to the resistance of Bcl-2 overexpressing cells to staurosporin. High levels of native TrxR did not protect from SecTRAPs. Thus, TrxR may be an important target for electrophilic anticancer agents, forming SecTRAPs that signal apoptosis through rapid caspase activation.

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Specific anti-STAT1 action of some natural polyphenols: Protective effect in inflammatory diseases

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Cyclooxygenase (COX) is widely considered as the molecular target of non-steroid anti-inflammatory drugs (NSAIDs). However, due to the harmful side effect frequently observed following chronic use, the development of new anti-inflammatory agents is the matter of many studies. Signal transducers and activators of transcription (STAT) are a family of nuclear proteins mediating the action of a number of cytokines. Among them, STAT1 plays a critical role in the signal transduction pathway of interferon-gamma (IFN-gamma) and growth hormone. STAT1 cascade is one major signaling pathway converting the IFN-gamma signal into gene expression, such as inducible nitric oxide synthase (iNOS), COX, vascular cell adhesion molecules (VCAM) and intercellular cell adhesion molecules (ICAM), critically involved in different pathologies correlated to the inflammatory process. We have recently reported data indicating that green tea extract (GTE), St. John's Wort extract and epigallocatechin-3-gallate (EGCG) exhibit a specific and strong anti-STAT1 activity which is independent of their acclaimed strong anti-oxidant activity. More recently, GTE has been shown to protect heart damage from ischemia/reperfusion in rats, suggesting that the protective effect of green tea might be correlated to its anti-STAT1 activity. The present work is aimed at providing data that STAT1 may potentially be claimed as a new molecular target of an anti-inflammatory treatment and that among polyphenols there are a number of anti-STAT1 substances.

Redox-sensitive transport of human thioredoxin-1

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Thioredoxin-1 (TRX) plays important roles in cellular signaling through redox control of cysteine residues in proteins 1). TRX was originally cloned as an adult T-cell leukemia (ATL)-derived factor (ADF) has cytokine-like activity from human T-cell leukemia virus-I (HTLV-I) transformed T-cells 2). TRX induced by several stimulations including oxidative stress is redox-sensitively released from various types of mammalian cells despite no typical secretory signal sequence, and regulates not only its own release, but also cellular redox states 3). One of the mechanisms is considered that TRX is internalized into the cells. We approached the detailed mechanism by using TRX derivative, in which Cys35 in the active site was replaces with serine used as a tool. TRX-C35S is bound on the cell surface and rapidly internalized in HTLV-I-transformed T cells as well as the activated T cells including Jurkat T cells stimulated by PMA/ionomycin. Moreover, the TRX-C35S is bound and localized in lipid rafts microdomain of plasma membrane in the activated T cells. Here, recent progress of the study of TRX internalization, especially focused on the correlation with lipid rafts will be discussed.

Thioredoxin binding proteins: Role for cancer protection

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Human thioredoxin (TRX) is a vital component of the thiol reducing system, regulating various cellular functions including apoptosis (1). The induction of TRX expression is observed in some tissues such as retinal pigment epithelial cells, leading to the protection against oxidative stresses (2, 3). Interaction between TRX and thioredoxin binding proteins is a novel mechanism of redox regulation. While apoptosis regulating kinase 1 (ASK1) is a regulator of apoptosis, thioredoxin- binding protein-2 (TBP-2) / vitamin D3 up-regulated protein 1 (VDUP1) has a character of tumor suppressor. The expression is down-regulated in several tumors and melanoma metastasis, suggesting a close association between the reduction and tumorigenesis. Loss of TBP-2 seems to be also an important step of human T cell leukemia virus type- I (HTLV-I) transformation (4,5), contributing to uncontrolled IL-2- independent growth in HTLV-I-infected T-cell lines (6). The expression of TBP-2 is induced by various stimulations and often shows a pattern reciprocal to that of TRX. TBP-2 is also implicated to play a crucial regulatory role in glucose and lipid metabolism as well as immune function. Here, recent progress of the study of TBP-2, especially focused on its role for cancer protection will be discussed.

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Glutathione peroxidases: Unexpected functions and expression control

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The family of glutathione peroxidases comprises at least 4 different selenoproteins. The individual glutathione peroxidases display clear differences in terms of tissue distribution, function, and regulation. The gastrointestinal GPx (GI-GPx, GPx2) has first been described to be exclusively expressed in the gastrointestinal system and, therefore, believed to function as a barrier against hydroperoxide absorption. It is, however, up-regulated in various types of cancer and can be induced in cancer cell lines by retinoic acid making a mere barrier function unlikely. Sporadic reports about an up-regulation of GI-GPx in array analyses performed from electrophile-treated cells or its increase prevented in Nrf2-knockout mice, led us to investigate whether GI-GPx might be Nrf2-regulated. In CaCo-2 cells, GI-GPx was induced by t-butyl hydroquinone (tBHQ) and sulforaphane (SFN), i.e., substances known to activate the "antioxidant response element" (ARE) via electrophilic thiol modification of Keap1 in the Nrf2/Keap1 system. Transcription factor analyses revealed a putative ARE in the promoter of GI-GPx. The functional significance thereof was demonstrated in HepG2 and CaCo2 cells by the activation of reporter gene constructs under the control of either the isolated ARE or the complete GI-GPx promoter by typical Nrf2 activators such as tBHQ, SFN, or curcumin, or by overexpression of Nrf2. The effects were reversed by mutation of the ARE within the promoter, by overexpressed Keap1, and by siRNA-mediated knock down of Nrf2. Binding of Nrf2 to the ARE sequence in gpx2 was corroborated by chromatin immunoprecipitation. With the identification of the GI-GPx gene as a target for Nrf2 we provide new insights into the transcriptional regulation of a selenoprotein.

Cu(II)-induced PI3-kinase signaling: Modulation of FoxO transcription factor activity

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Exposure of various types of mammalian cells to Cu2+ resulted in a dose- and time-dependent activation of the anti-apoptotic kinase Akt/protein kinase B, starting at concentrations as low as 3 μM. The pathway was also activated by Zn(II) and, to a lesser extent, by Cd(II). Inhibitors of phosphoinositide-3-kinase (PI3K) completely blocked activation of Akt by Cu(II), indicating a requirement of PI3K for Cu(II)-induced activation of Akt. Indeed, cellular PI3K activity was strongly enhanced after exposure to Cu(II). Activation of Akt was accompanied by phosphorylation of downstream targets of Akt, including glycogen synthase kinase-3 as well as members of the FoxO family of transcription factors, FoxO1 (FKHR), FoxO3a (FKHR-L1) and FoxO4 (AFX). This phosphorylation was prevented by the PI3K inhibitors wortmannin or LY294002. Phosphorylation of FoxO transcription factors by Akt results in their inactivation and shuttling from the nucleus to the cytoplasm. Stimulation of cells with metal ions led to a rapid relocalisation of a FoxO1-GFP fusion protein from the nucleus to the cytoplasm. This effect was attenuated in the presence of wortmannin and was not seen with a FoxO1-GFP mutant devoid of Akt phosphorylation sites, implying that Cu(II) or Zn(II) activate PI3K, Akt as well as induce the Akt-dependent phosphorylation of FoxO1, resulting in its subcellular relocalisation. As FoxO transcription factors are important regulators of the expression of key proteins in cell cycle control (p27kip), DNA repair (Gadd45a) or antioxidative defense (e.g. catalase, manganese superoxide dismutase), they likely play a crucial role in the cellular response to a heavy metal stress.

The cytoskeleton in the modulation of cell signaling by zinc

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Developmental zinc (Zn) deficiency is associated with severe neurological alterations, although the mechanisms involved are still unknown. We have observed that a decrease in cellular Zn induces cell cycle arrest and apoptotic death in neuroblastoma cells. Zn deficiency also leads to a rapid neuronal increase in reactive oxygen species (ROS) that modulate oxidant-responsive signaling pathways (NF-κB, AP-1, NFAT). Other cell signals remain unaffected (CREB, OCT-1). In Zn deficiency: 1) ROS trigger the activation of MAPKs p38 and JNK leading to AP-1 activation and a high transactivation of AP-1-dependent genes, and 2) ROS activate the initial steps of NF-κB and NFAT signaling cascades. However, the expression of NF-κB- and NFAT-dependent genes is inhibited due to an impaired nuclear translocation of the active transcription factors. The altered polymerization and structure of microtubules associated with the decrease in cellular Zn, and possibly to the associated increase in ROS could be involved in the impaired cytosolic transport and nuclear import through the nuclear pore of the active NF-κB and NFAT. This mechanism is supported by the similar effects exerted by cytoskeleton-disrupting chemical and physical conditions. In summary, ROS and the cytoskeleton are involved in Zn deficiency-induced altered modulation of AP-1, NF-κB and NFAT in Zn deficiency. This can in part underlie the adverse effects of Zn deficiency on the development and function of the nervous system.

Supported by grants from CONICET (PIP 02120), Argentina, the University of California and NIH (HD 01743), USA

Molecular mechanisms involved in the adaptive response to oxidative stress

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A major target of our research group is to study the molecular mechanisms involved in the redox control of gene expression, cell signalling and cellular senescence (1, 2). Preliminary evidence on the functional role of two genes involved in the cell adaptive responses to oxidative stress is here presented. aos-2 human osteosarcoma cells were chronically adapted to grow in mild oxidizing conditions induced by diethylmaleate (DEM), a GSH depleting agent. Gene expression profiles of DEM-adapted and untreated cells were compared using a differential display technique. Among the genes whose expression was up-regulated upon DEM treatment, we focused on: i) an Expressed Sequence Tag (EST) coding for a multi-tract transmembrane protein, whose function is as yet unknown; ii) mitochondrial Hsp75 (TRAP1), that may exert an active role in oxidants-induced apoptosis. The functional role of both genes is under investigation: stable transfection of expression vectors for each protein results in an increased resistance to oxidative DNA damage and apoptosis. Since resistance to oxidative stress is regarded as an important mechanism involved in the resistance to chemotherapeutic drugs, we assayed mRNA levels of these two genes in a colon carcinoma cell line resistant to fluorouracil (5-FU). The expression of both transcripts was highly induced in 5-FU resistant cells. Further studies on the functional role of the 3'UTR regions in the regulation of Hsp75 expression are in progress. hese data may contribute to a better understanding of the role of these two genes in the adaptive response to oxidative stress and, ultimately, to the identification of new molecular mechanisms involved in chemoresistance.

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SESSION V
BIOLOGICAL AND PHYSIOLOGICAL EFFECTS OF FLAVONOIDS AND POLYPHENOLS

Bioavailability of flavonoids and polyphenols

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Polyphenols are naturally occurring dietary compounds with several effects on health biomarkers. Although they have antioxidant activity, they have numerous other reported biological activities. In order for the polyphenol to be biologically active, it must be bioavailable i.e. reach the site of action. There are many human studies on bioavailability of polyphenols. Based on plasma concentrations (area under the curve) after a single dose, the most bioavailable polyphenols are: isoflavones > flavonols (quercetin) > flavanones (hesperidin, naringenin) > catechins > anthocyanins = procyanidins. The mechanism of absorption, and the relationship between absorption and biological effect, will be discussed.

Chocolate and wine flavanols in hypertension: The search for reliable biochemical mechanisms

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Flavanols and their oligomers (procyanidins) are naturally occurring plant compounds, preferentially present in chocolate and wine. Dietary intervention studies in humans and rats indicate that flavanol-rich foods, i.e. wine, tea, and chocolate may exert blood pressure lowering effects. In parallel, recent reports have shown that blood pressure can be regulated by ROS and NO production. The free radical scavenging properties of flavonoids are often claimed as potential contributors to those antihypertensive effects, but the very low concentration of flavanols, procyanidins and their metabolites in blood, do not support that antioxidant action. We propose that flavanols and procyanidins present in chocolate and wine can interact with cell membranes, inhibit angiotensin converting enzyme activity (ACE), and consequently modulate the steady-state levels of oxidants and NO through angiotensin II-dependent mechanisms. This hypothesis is based on results showing that: a) flavanol and procyanidins interact with membranes, and also inhibit competitively ACE activity in vitro; b) flavanols and related procyanidins prevent oxidation and NFκB activation in cultured cells; and c) ACE inhibitors modulate oxidative stress and NO production in humans and rats. If the proposed mechanisms are operative in vivo, they will provide a new insight on how the moderate consumption of chocolate, wine, and other flavanol-containing foods could contribute to optimize health.

This work was partially supported with grants from the ANPCyT, Argentina (PICT 01-08951); and Mars Inc., USA.

Health effects of polyphenols: Is it a matter of single nutraceuticals or a diet as a whole?

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Epidemiological evidence agrees with grandmother wisdom in suggesting an increased intake of fruit and vegetables. Unraveling the mechanisms would render this recommendation fully sound and facilitate a further optimization of nutrition. It is generally accepted that antioxidant polyphenols play the above protective role. However, due to the actual concentrations reached in body fluids, the relevance of an antioxidant mechanism has been questioned. As an alternative mechanism, flavonoids have been suggested to be involved in controlling cell response to challenges, usually oxidative and often related to nutrition. This effect is compatible with in vivo concentrations and the control of pro-inflammatory nuclear factors NFκB and AP1 is seen as protective in respect chronic degenerative diseases. Consistently, the unbalance between different components of a meal (pro-oxidants vs. reducing), described as "post-prandial oxidative stress", brings to the focus the importance of taking the "protective antioxidant" in the same meal when "oxidative challenges" are taken. This concept also rescues the antioxidant function of flavonoids, when considering the inhibition of lipid peroxidation taking place in the food before absorbtion. As an example, wine procyanidines taken at a meal minimize the post-prandial increase of lipid oxidation products in plasma, thus preventing the formation of the atherogenic, misfolded LDL. In conclusion, the above mechanisms emphasize the notion that protective elements must be taken as components of the meal, and again, this fits remarkably well the grandmother wisdom.

Green tea and oatmeal for breakfast

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Epidemiological and experimental studies indicate that the consumption of oats and green tea has several health benefits. Due to their high soluble fiber content, oats reduce cholesterol levels and green tea due to its catechins contributes to the prevention of CVD and cancer. Oats also contain unique polyphenols, avenanthramides (Avns), which we discovered they may prevent atherosclerosis through inhibition of several pro-inflammatory cytokines (IL-6, IL-8, MCP-1) and adhesion of monocytes to endothelial cells (EC). Here, we report the molecular mechanisms by which Avns also inhibit vascular smooth muscle cell (SMC) proliferation and increase nitric oxide (NO) production, both of which are important in the development of atherosclerosis. Avns arrest the cell cycle of SMC in G1 phase through inhibition of pRb phosphorylation. This is accompanied by a decrease in cyclin D1 expression and an increase in the expression of p53 and $p21^{cip1}$, the cyclin-dependent kinase inhibitor. The increase in NO production by SMC and EC was associated with Avns up-regulation of mRNA expression for eNOS. We have also recently discovered a unique mechanism by which green tea catechin (EGCG) may inhibit angiogenesis and contribute to its anticancer effect. We found that EGCG inhibition of angiogenesis is mediated through inhibition of phosphorylation of VEGF receptor and VE-cadherin and by inhibition of activity of PI_3-kinase activity leading to inhibition of VEGF receptor complex (VEGF/VEGFR; β-catenin; VE-cadherin; PI_3-kinase) formation, which is necessary for downstream Akt phosphorylation, NF-κB activation, and IL-8 production during angiogenesis. These in vitro cell culture studies provide supporting evidence for the potential health benefit of daily consumption of oats and green tea.

Cocoa flavanols modulate the prooxidant-antioxidant balance in human plasma by lowering F2-isoprostane concentrations

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Background: Flavan-3-ols are potent antioxidants *in vitro*, but convincing evidence for this action *in vivo* is lacking. Plasma F_2-isoprostanes are a suitable parameter for non-enzymatic lipid peroxidation *in vivo*. We examined whether a flavanol-rich cocoa drink attenuates oxidative stress-mediated increase in plasma F_2-isoprostanes.

Study Design: With 20 healthy male volunteers, the *in vivo* response to ingestion of low-flavanol (LFCD; 14 mg/100 mL) *vs.* high-flavanol cocoa drink (HFCD; 187 mg/100 mL) was assessed in a comparative randomized double-blind crossover study. Venous blood was drawn immediately before and 2, 4, and 6 h after intake. With 10 individuals the treatment was combined with strenuous exercise by treadmill. Total (esterified plus non-esterified) 8-iso-$PGF_{2\alpha}$ and 9α,11α-$PGF_{2\alpha}$ were analyzed by GC/MS.

Results and Conclusion: LFCD caused a slight increase in the mean plasma concentrations of the two F_2-isoprostanes 2 and 4 h after intake, which may be attributable to postprandial oxidative stress and did not occur with HFCD. A comparison of the effect of LFCD *vs.* HFCD revealed significant decrease in the sum of F_2-isoprostanes 2 and 4 h after intake ($P < 0.01$, ANOVA) when the intake was combined with physical exercise. Other parameters of plasma prooxidant/antioxidant status did not reveal significant changes. It is concluded that a high intake of flavanols controls the plasma level of F_2-isoprostanes.

Transcriptome and protome analysis of human endothelial cells supplemented with the soy isoflavone genistein

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Endothelial cells are primary targets for proatherosclerotic stressors such as oxidized LDL (ox-LDL). The isoflavone genistein, on the other hand, is suggested to prevent a variety of processes underlying atherosclerosis. By analyzing the proteome of endothelial cells, we show, that genistein reverses ox-LDL-induced changes of the steady-state levels of several proteins important in atherogenesis (Fuchs et al. 2005). Furthermore, several lines of evidence suggest that genistein has beneficial effects with regard to blood pressure. The biological action of genistein may be at least in part attributed to its ability to affect cell signalling and response. To examine the transcriptional response to genistein on primary human endothelial cells (HUVEC), we applied DNA array technology. The alteration of the expression patterns of individual transcripts was substantiated using either RT-PCR or Northern blotting. Genistein significantly affected the expression of genes encoding for proteins involved in the regulation of the vascular tone such as endothelin converting enzyme 1, endothelin-2, estrogen related receptor alpha and atrial natriuretic peptide receptor A precursor. Isoflavones undergo extensive metabolism in the gut and in the liver. Recent data indicate that sulfation of genistein significantly decreases its antioxidant activity and its impact on endothelial function (Rimbach et al., 2005).

Fuchs et al., J Proteome Res. (2005): 4(2),369-76.
Rimbach et al., Biochim. Biophy. Acta (2004): 1689,66-74.

Polyphenols from wine and the diet: eNOS and metabolic syndrome

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Results from our and other laboratories, support the hypothesis that the improvements in cardiovascular risk factors after red wine consumption, such as plasma lipids, haemostatic mechanisms, endothelial function, and antioxidant defences, are mediated by endothelial nitric oxide synthase (eNOS). Other genes are involved, but eNOS would be a constant feature. Also mediterranean diets, via plant phenolic antioxidants and ω-3 fatty acids at least, enhance endothelial function. The metabolic syndrome, a cluster of risk factors associated to cardiovascular disease, affects 10-30% of the population. Its diagnosis is made (NCEPATP III) when three or more risk factors, from among abdominal obesity, high plasma triacylglycerols, low plasma HDL, high blood pressure, and high fasting plasma glucose are present. The molecular mechanisms responsible for the metabolic syndrome are unknown. The recent finding that eNOS knockout mice present a cluster of cardiovascular risk factors comparable to those of the metabolic syndrome, suggests that defective eNOS could originate human metabolic syndrome. These mice are hypertensive, insulin resistant and dyslipidemic. Further support in humans comes from the association of eNOS polymorphisms with insulin resistance and diabetes, with hypertension, with inflammatory and oxidative stress markers, and with albuminuria. Therefore, increased function of eNOS should reduce metabolic syndrome incidence and its consequences. Red wine and mediterranean diets, since they enhance eNOS function, should be considered as potential tools for the control of the metabolic syndrome.

ALEX SEVANIAN MEMORIAL LECTURE

Dietary polyphenols to limit oxidative stress and improve human health
A future open but still paved with much uncertainty

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Dietary polyphenols (PPs) are considered as antioxidants due to their capability to scavenge free radicals, boost antioxidant defenses or improve the status of oxidative stress biomarkers. However, the evaluation of their health effects is difficult due to (i) their high chemical diversity and wide distribution in food, (ii) limitations of experimental studies often carried out at non-nutritional doses or with PP virtually absent in human tissues, and (iii) lack of firmly established links between oxidative stress biomarkers and health. Progress will notably arise from a better knowledge on the distribution of PPs in food and on a more accurate determination of their dietary intake. A new comprehensive food composition table for PPs is under construction. It should allow to better assess the associations between PP exposure and health in epidemiological studies, and also to prioritize research on PPs showing the highest biological activities at dietary intake levels. Progress will also arise from an improved knowledge on PP metabolism and on the distribution of the metabolites in the different target tissues. Some recent progress on both flavonoid and phenylpropanoid metabolism will be illustrated and their practical consequences for future research emphasized. The perspectives offered by the 'omic' technologies will be discussed. They may lead to the discovery of new biomarkers of effect for PPs beyond the common biomarkers of oxidative stress. The comparison of the metabolic profiles of the different PPs may allow to identify common targets or mechanisms of action for PPs in general as well as specific targets for particular PPs. A clearer definition of antioxidants should arise from such studies.

Session VI
Oxidants and Antioxidants in Neurodegeneration and Aging

Redox regulation of heat schock protein expression in aging and neurodegenerative disorders: Role of vitagens and modulation by acetylcarnitine

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Oxidative damage plays a crucial role in the brain aging process. Induction of heat shock proteins (HSPs) is critically utilized by brain cells in the repair process following various pathogenic insults. We have recently focused our research on the role of acetylcarnitine (LAC) in the defense mechanisms against cellular stress and neurodegeneration. In the present study we investigated mRNA expression and protein synthesis of Hsps and the oxidant status in adult (12 months old) and senescent (28 months old) rats, and the effect of LAC (150 mg/kg per day) treatment given for 4 months to senescent rats. mRNA and protein synthesis of Hsps increased in senescent rats compared to adults in all brain regions examined; the maximum increase was observed in the hippocampus followed by cerebellum, cortex, and striatum. Hsps increase was associated with significant increase in GSSG/GSH ratio, carbonyls and HNE content. Interestingly, treatment with LAC resulted in a further increase of heme oxygenase-1 (HO-1), Hsp70, and mtSOD expression, and a decrease of GSSG/GSH ratio, HNE and protein carbonyl contents, in the hippocampus, striatum, cortex and cerebellum. These results were also confirmed by in situ hybridization experiments. We used also a parallel redox proteomic approach to identify the proteins that are oxidized in aged rat brain and those proteins that are protected by LAC treatment. These findings are relevant to potential pharmacological strategy for protection of functional decline associated with age-related impairment, and neurodegenerative diseases.

Intracellular toxicity of β-amiloid peptide: Role of mitochondrial oxidant generation

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Alzheimer's disease is an age-related disease of multifactorial origin. Extracellular deposits of β-amyloid plaques are a consistent finding in post-mortem examination of these patients. It is generally accepted that β-amyloid peptide causes neuronal lesions that are at least in part responsible for cellular damage in this disease. There is, however, growing evidence of intracellular toxicity of β-amyloid peptide. The aim of this work was to examine the possibility that β-amyloid directly increases oxidant production by mitochondria, thus activating the mitochondrial pathway of apoptosis (mediated by the release cytochrome c). Since the incidence of Alzheimer's disease is higher in women than in men the effect of gender of β-amyloid peptide increase in oxidant production by mitochondria has also been examined. The most sigi¡nificant results which will be presented are: 1. β-amyloid peptide increases peroxide production by brain mitochondria from young males or from old females but not from young females. 2. The reverse, non-toxic β40-1 peptide does not cause any effect on peroxide production by mitochondria. 3. β-amyloid peptide increases nitration of mitochondrial proteins. 4. β-amyloid peptide increases aggregation of brain mitochondria from young males or old females, but not from young females. 5. Cytochrome c release by mitochondria is increased by β-amyloid peptide in brain mitochondria from males but not in those from females. 6. Glutathione prevents the deleterious effects of β-amyloid peptide on mitochondria. The possible pathophysiological importance of these results will we discussed.

Nitric oxide dynamics in hippocampus following activation of NMDA and AMPA receptors

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The current understanding of •NO activity as a diffusible intercellular messenger in the brain implies the formation of a "sphere of influence", affecting a volume of tissue containing many neurons irrespective of functional connections through synapses. Thus, the rate and pattern of local •NO concentration change (concentration dynamics in time and space) establishes its actions at any location. The measurement of •NO dynamics during functionally induced changes in hippocampus by activation of the glutamate receptors has been a goal of much interest as the activation of these receptors is essential to signaling pathways that underlie neuronal plasticity and development, but also triggers neurodegeneration that occurs in senescence and disease. Using selective microsensors we firstly determined the diffusional spread of endogenously-generated •NO in CA1 subregion of hippocampal slices. Simultaneously following •NO and PO_2 profiles permitted to correlate the utilization O_2 with •NO concentration. In addition to NMDA receptors, the synthesis of •NO is sensitive to a selective stimulation of the glutamate AMPA receptors. In both cases cells were able to efficiently respond to high •NO concentrations bringing it to very low nM levels. Finally, the production of NO via glutamate receptors can be differently modulated (*e.g.* by ethanol) via NMDA and AMPA receptors. These observations provide a quantitative and basic framework for the understanding of •NO as an intercellular messenger in the hippocampus.

Supported by FCT: POCTI/BCI/42365/01

A two-hit hypothesis for the pathogenesis of Alzheimer's disease

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Since oxidative stress is an early event in Alzheimer disease (AD), occurring proximal to the development of hallmark pathologies, it is thought by many investigators to play an important role in the disease. Studies into the cause of oxidative stress in AD show that interactions between abnormal mitochondria and disturbed metal metabolism play a key role. Counter to popular dogma, amyloid-β deposition and hyperphosphorylated tau actually appear to function as downstream compensatory responses that lessen oxidative stress and ensure that neuronal cells do not succumb to oxidative insult. Despite its early role, oxidative stress alone does not account for the totality of either the etiology or the pathogenesis of the disease. In this regard, recent studies by our group, and others, show that aberrant mitogenic alterations, like oxidative stress, play an important early role in the disease process. Interestingly, while either oxidative stress or abnormalities in mitotic signaling can independently serve as initiators, both processes appear to be necessary to propagate disease pathogenesis. Therefore, the initiation and progression of AD, like cancer, appears to require "two hits" – oxidative and mitogenic.

Work in our laboratories is sponsored by the NIH, the Alzheimer's Association, and by Philip Morris USA Inc. and Philip Morris International.

Changes in hippocampal neurogenesis and function in drug addiction

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Severe cognitive impairment consistently occurs in chronic alcoholism regardless of the presence of associated thiamine deficits (Korsakoff Syndrome), and it includes progressive and severe anterograde learning deficits, implicating impairment of hippocampal circuits. Most of the previous published data suggest a role for oxidative or nitrosative stress in ethanol-induced nervous system damage. Moreover, ethanol is able to impair learning abilities in adult mammal brain, a process suggested to be directly related with neurogenesis. Ebselen, a synthetic compound with antioxidant properties is able to prevent ethanol-induced impairment of neurogenesis in adult rats, and our data demonstrate its ability to prevent biochemical alterations, long term potentiation and learning abilities in hippocampus of chronic alcoholic adult rats. The hippocampal concentrations of glutathione and of malondialdehyde are decreased and increased, respectively, in alcohol-treated animals, and do not differ from control values in the alcohol and ebselen group. LTP in hippocampal slices from ethanol-treated animals is prevented, when compared to controls, and occurs with similar profile to control animals in the group treated with ethanol and ebselen. The learning ability of these rats was tested with the Morris water maze test. Escape latencies were higher in ethanol-treated rats than in control animals or the ones treated with ethanol and ebselen. In conclusion, oxidative mechanisms are related to the hippocampal effects of ethanol in adult rats, as demonstrated by the protective effect of ebselen. A similar approach has been used with cocaine and ecstasy in rats with results showing also impairment of hippocampal neurogenesis. Experiments with ebselen are now being performed.

Mitochondrial glutathione: Regulatory roles in mitochondria

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Glutathione (GSH) plays a key role in detoxification of reactive oxygen metabolites (peroxides) and is an important regulator of protein redox status. There exist two major GSH pools in cells, a cytoplasmic and mitochondrial pool; the latter is important in detoxification of hydrogen peroxide produced by the electron transport chain. Mitochondrial GSH is considered vital for cell survival. Cell necrosis caused by GSH-depleting agents (*e.g.*, diethylmaleate, acetaminophen) is believed to occur only after mitochondrial GSH depletion, with cytoplasmic GSH depletion being less consequential. Alterations in mitochondrial GSH levels have profound effects on hydrogen peroxide release by mitochondria and activity of redox sensitive mitochondrial proteins such as aconitase. GSH is important in modulating aconitase activity when challenged by oxidants such as peroxynitrite. The redox status of mitochondrial GSH appears to be determined by the levels of mitochondrial substrates. In the absence of substrates, significant glutathionylation of mitochondrial proteins can occur. The glutathionylation of proteins may play an important regulatory role in mitochondria during times of oxidative stress.

Mitochondrial dysfunction in the hippocampus and in the striatum body

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Brain neurons group their neuronal bodies in 65 bilateral nuclei and in the cortex area, which are macroscopically recognized as grey matter. Neuronal nuclei have an intense aerobic metabolism and an important fraction of the cytosolic space is occupied by mitochondria. Rat hippocampus and striatum body are brain areas that provide enough tissue to isolate mitochondria. Hippocampal mitochondria showed rates of state 3 respiration, with succinate and malate/glutamate as substrates, of 80-120 and 70-100 ng-at O/min.mg protein, and with respiratory controls of 3.1-3.6 and 3.8-5.3. Hiperammonemia (4 times the normal values), produced by portal hypertension, decreased state 3 respiration to 58% and mitochondrial nitric oxide synthase (mtNOS) activity to 46% associated with normal ultrastructure in the neurons and with ultrastructural damage in astrocyte and endothelial cell mitochondria. Aging, from 28 to 56 wk of rat age, decreased the state 3 respiration of hippocampal mitochondria by 40%; an effect that was 70% prevented by high dose-vitamin E supplementation in the diet (5 g vit E/kg food). Striatal mitochondria showed succinate-supported rates of state 3 respiration of 120-140 ng-at O/min.mg protein with respiratory controls of 3.3-4.0. A model of Parkinson's disease (rotenone, 2 mg/kg/d i.p., 60 days) in rats reproduced the neurological symptoms (ataxia) and produced significant decreases in striatal tissue and mitochondrial state 3 oxygen uptake (24-35%) and in mtNOS activity (25%).

SESSION VII
CLINICAL STUDIES ON ANTIOXIDANTS

Protection of DNA damage by antioxidants: Potential relationship with two French epidemiological studies, EVA and SUVIMAX

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In two recently realized large epidemiological studies in France we observed a strong relationship between the occurrence of age-related diseases and deficient antioxidant status. The EVA study followed the decline in cognitive function of 1300 healthy people 65 years old. A faster decrease in memory was observed after 5years, in those of the subjects with the higher peroxidation and lower selenium or beta carotene serum level at the entrance. The SUVIMAX study demonstrated a higher risk of cancer for subjects with a low beta carotene status. 8 years of supplementation with a mixture of antioxidant increased survival rate and decreased cancer risk by 35% in men with no effect in women. But women presented before supplementation a spontaneous nutritional status two time more rich in beta caroten and ascorbate. The effect of beta carotene was stronger in men with a basal low status. Moreover supplementation with antioxidant increased the occurrence of skin cancer in women not in men. Demonstrating that too much antioxidant are not necessary and harmfull in subjects with a sufficient nutritional status. The effect of antioxidant may be related to antioxidant-induced protection of DNA damages. DNA damages when non repaired present an apoptotic effect on neural cells and when badly repaired are the first step of carcinogenesis. We demonstrated the protective effect of antioxidant on DNA damages in animal and cell cultures. But we failed to demonstrate this effect in SUVIMAX. Since that date we dispose now in the lab of new biological indicators of DNA that appears very relevant to oxidative stress. So we push now a new study named SUVIMAXII to demonstrate the responsibility of DNA damages in cognitive decline and cancer occurrence.

Vitamin E and respiratory infections in the elderly

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Respiratory infections are prevalent in the elderly, resulting in increased morbidity, mortality, and utilization of health care services. Contributing to the increased incidence of infection with age is the well-described decline in immune response. Recently, however we have shown that the aged host also promotes evolution of a virulent viral species from a non-virulent species. These findings propose a new host-virus paradigm for studies of viral infection in the aged. Nutritional status is an important determinant of immune function. We have shown, in double-blind, placebo controlled trials that vitamin E supplementation significantly improved immune response, including DTH and response to vaccines. Furthermore, subjects receiving E in the 6-month trial had a 30% lower incidence of infectious diseases. These findings were supported by studies in animal model of influenza as well as secondary bacterial infection. In addition, recently, we conducted a randomized double-blind, placebo controlled trial to determine the effect of 1-year supplementation with 200 IU/day vitamin E on incidence and duration of respiratory infections in 617 elderly nursing home residents. Vitamin E supplementation significantly reduced the risk of acquiring upper respiratory infections in elderly nursing home residents. In addition, a significant reduction in incident rate of common colds and the number of subjects who acquire a cold was observed. A non-significant reduction in the duration of colds was also observed. Further studies are needed to determine the mechanism of vitamin E induced increase resistance to viral infections. In light of our new findings on the effect of aging on viral evolution, these studies should focus on immune and non-immune related host factors.

Oxidative stress and mild cognitive impairment

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A large body of evidence supports the role of oxidative stress in aging, Alzheimer's disease (AD) and in cerebrovascular disease. Aging is the major risk factor for AD. A vascular component might be critical in the pathophysiology of AD, but there is a substantial lack of data regarding the simultaneous behavior of peripheral antioxidants and biomarkers of oxidative stress in AD and vascular dementia (VaD). Furthermore, a large body of attention is being recently given to the evaluation of oxidative stress in pre-AD conditions like mild cognitive impairment (MCI). Independent of their nature – vascular or degenerative – MCI and dementia are associated with the depletion of a large spectrum of antioxidant micronutrients and with increased protein, lipid and DNA oxidative modification. This might be relevant to the pathophysiology of dementing disorders, particularly in light of the recently suggested importance of a vascular component in AD development.

Antioxidants for the tratment of cardiovascular disease: Is there a future?

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Oxidative stress seems to play a key-role in the pathogenesis of atherosclerosis. Agents that prevent LDL from oxidation have been shown in a range of in vitro and animal models to reduce the development and progression of atherosclerosis. Among these, the antioxidant micronutrients, including vitamin E have gained wide interest because of the potential for prevention of atherosclerotic vascular disease in humans. In the last decade many trials with antioxidants have been planned in patients with cardiovascular disease but the results are equivocal. The reason for the disappointing findings is unclear but one possible explanation is the lack of identification criteria of patients who are potentially candidates for antioxidant treatment. Data reported so far will be analysed to determine whether they clearly support the premise that patients at risk of cardiovascular disease may be candidates for antioxidant treatment.

POSTERS

Low antioxidant status in the critical care patient

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1. Introduction. Critical disease implies risk of death, in which the formation of reactive organ species (ROS) are induced. The organism possesses non-enzymatic enzymes that form an endogenous line of defence. The total antioxidant capacity (AOC) can be compromised by the an inadequate supply of nutrients. *2. Objective*. To assess the AOC in the critical patient and its evolution during one week, examining its relationship with endogenous and dietary antioxidants. *3. Material*: 40 patients over 18 years old admitted to the ICU of the University teaching hospital who were receiving artificial nutrition and were in the ICU for at least one week. *Methods*: The measurements (AOC, albumin, uric acid, bilirubin and total proteins)were made using standard colorimetric techniques. The mean intake of antioxidant nutrients was calculated. The means were analyzed using the Student test and Pearson's correlation coefficient ($P < 0.05$). *3. Results*. Antioxidant capacities were significantly lower after one week of ICU stay 1.421 (ng/mg prot) and 1.19 (ng/mg prot)respectly. The asociation between low AOC and low bilirrubin and uric acid was significant ($r^2 = 0.355$; $p = 0.031$; $r^2 = 0.388$; $p = 0.016$ respectly). Also, who received, a contribution adapted of antioxidant vitamins to recommendation, did not make worse the parameters of oxidative stress ($P = 0{,}003$). *4. Conlusions*. This study show a significant reduction in antioxidant capacity in the patients studied. The constant situation of metabolic stress suffered by the critical care patient may alter endogenous defence mechanisms and exacerbate oxidative stress in these patients.

PseudoEC-SOD
A functional mimic with potential protecting effects

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Many degenerative processes and diseases such as aging, reperfusion damage of ischemic tissue and inflammatory actions have been coupled to the action of oxygen radicals. One important protective actor is the superoxide dismutases (SODs), which catalyzes the dismutation of superoxide radicals. Interest has therefore evolved in the therapeutic potential of SOD-enzymes. Most work has focused on the cytosolic SOD, even though it is not normally located in the extracellular space, and has a plasma half-life of only seven minutes. The optimal SOD to use as a therapeutic agent would be EC-SOD, which during evolution has been designed for extracellular function. The enzyme is normally associated to heparan sulphate proteoglycans on the cell surfaces and has a half-life of 20 hours in circulation. The evaluation of the potential therapeutic value of EC-SOD has been hampered by failure of large-scale production. To overcome this problem a fusion protein, mimicking human EC-SOD, was constructed. The characteristics of this PseudoEC-SOD closely resemble those of human EC-SOD. In this immunohistochemical study we show that PseudoEC-SOD binds to cell surfaces of human tissue in vitro, in a very similar way as native EC-SOD does. We also tested PseudoEC-SOD in an ischemia-reperfusion model in isolated rabbit hearts, and the preliminary results indicate a protective effect.

A decrease in cellular zinc increases cell oxidants partially through NADPH oxidase

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Several studies have shown that a condition of zinc (Zn) deficiency increases the production of cell oxidants such as reactive oxygen (ROS) and nitrogen species. However, the mechanisms involved in the high levels of cellular ROS are still unclear. The objective of this study was to test the hypothesis that a decrease in cellular Zn can lead to an increased ROS production through a mechanism that involves NADPH oxidase. For this purpose, human IMR-32 neuroblastoma cells were cultured (2-6 h) in control (C) or Zn-chelated media containing 1.5 (1.5 Zn) or 15 μM (15 Zn) Zn. Global cell oxidants, measured with DCDCDHF, H_2O_2 production, measured with Amplex Red, and the activation of the H_2O_2-responsive transcription factor AP-1 were determined. After 2-6 h incubation, significantly higher levels of oxidants (DCDCDHF) were found in the Zn deficient (1.5 Zn) (30-40%, $p < 0.005$) compared to C and 15 Zn cells. This increase was reduced ($p < 0.001$) when cells were incubated simultaneously in the presence of 0.5 μM DPI, a NADPH oxidase inhibitor. Similar results were observed when H_2O_2 was determined. We next evaluated the possible short-term effects of Zn deficiency-induced increase in H_2O_2 on the activation of AP-1. A significant increase in AP-1-DNA binding activity (40%) and in the transactivation of an AP-1-driven gene (p-AP1-Luc) (2-fold) was observed after 2 and 6 h, respectively, of incubation in Zn deficient media compared to C or 15 Zn groups. Results indicate that Zn deficiency could increase ROS production and trigger oxidant-responsive cell signals partially through a mechanism involving NADPH oxidase. Supported by grants from the University of California and NIEHS-CEHS, USA.

Pomegranate wine has a greater protection capacity than red win on LDL oxidation

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Although there is a large body of evidence on the main role of red wine in protection of LDL against oxidation, there is little data on the role of pomegranate juice that has high phenolic content. Considering the possible importance of pomegranate wine as antioxidant and in order to could made a comparison between red and pomegranate wine, we conducted this study. The phenol levels of red and pomegranate wine (4850 mg/l GAE and 815 mg/l GAE, respectively) were in accordance with their total antioxidant activity (39.5% and 33.7%, respectively). Both wines decreased the LDL-diene levels in the following 30 min incubation period compared with control (145 ± 3 μmol/mg LDL protein). However, pure pomegranate wines demonstrated higher antioxidant effect ($p < 0.01$) on diene level (110 ± 5 μmol /mg LDL protein) than pure red wines (124 ± 3 μmol/mg LDL protein). In conclusion, we suggested that pomegranate wine has potential protective effect toward LDL oxidation and it may be a dietary choice for persons who prefer fruit wines.

Mass spectrometric and conformational analyses of actin covalently modified by acrolein

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Recent proteomic evidences indicate a significant increase in actin carbonylation in several pathological situations. Actin, being characterized by several superficial nucleophilic sites (Cys, His and Lys residues) (1), can be a target for Michael adduction by reactive α,β-unsaturated aldehydes. We tested the ability of acrolein (ACR) to react with actin in in vitro conditions. ESI-MS analysis of G- and F-actin treated with ACR (1:1 to 1:50 mol/mol) showed the dose-dependent decrease of native protein (41872 Da), concomitant to the formation of 2 main protein adducts consistent with the Michael addition of one and two ACR residues. In order to identify the site(s) of modification, ACR-treated and native actin were stabilized with NaBH4, enzimatically digested with trypsin and the resulting peptides subjected to LC-ESI-MS/MS in data-dependent scan mode. Peptide mass mapping of native actin provided approximately 80% of sequence coverage. ACR was found to react with Cys374 which, as demonstrated by computational analysis, is characterized by a significant accessible surface and substantial thiol acidity due to the particular microenvironment. Moreover, ACR was found to adduct His residues, mainly His87 and in a lower extent His40. Molecular dynamics and modeling analyses indicates that actin carbonylation caused structural distortions that can explain the changes in the polymerization rates (see abstract by Dalle-Donne et al.).

(1) G. Aldini et al., J. Mass Spectrom. 2005 June (on line)

H_2O_2 diffusion across biomembranes: Is it slow, fast, or free?

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H_2O_2 diffuses across biomembranes much faster than other reactive oxygen species. This led to the concept that H_2O_2 can exert its biological effects far away from the local of production, and the dogma that H_2O_2 diffusion across biomembranes is not rate-limiting (i.e., is free) became progressively established. Here we analyze H_2O_2 diffusion from a quantitative point of view and confront the latest experimental data with the established dogmas. It can be shown that: (*1*) H_2O_2 diffusion is relatively slow; *e.g.*, it is about one order of magnitude slower than water. (*2*) In spite of this, H_2O_2 diffusion across biomembranes is fast enough in order to reach steady-state dynamical equilibration between different cellular compartments in the order of seconds. So, the concept that H_2O_2 can exert its effects away from its local of formation holds. (*3*) For most cell types studied, H_2O_2 diffusion is slower than the rate of H_2O_2 removal by antioxidant enzymes. That is, H_2O_2 diffusion is partially rate-limiting and, therefore, gradients are formed when the source of H_2O_2 is separated from the sink by a biomembrane. (*4*) The permeability of biomembranes to H_2O_2, and consequently the magnitude of the gradients, is subjected to regulation by H_2O_2 and other agents, establishing a new area for the biological action of H_2O_2.

Acknowledgements: Work supported by grant POCTI/BCI/42245/2001 and by fellowship BPD/11487/2002 (to FA), FCT/FEDER – Portugal.

Diallyl disulfide induces transient cell cycle arrest but not apoptosis in AGS adenocarcinoma gastric cells

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We have previously observed that diallyl disulfide (DADS), an oil soluble derivative of garlic, was able to efficiently induce apoptosis in neuroblastoma cells by a ROS-dependent pathway. In this study, we report that DADS was not cytotoxic to adenocarcinoma gastric AGS cells where, instead, it induced a prominent cell cycle arrest in G2/M phase at 6-12 h of treatment. This condition was followed by the re-entry in the cell cycle without commitment to apoptosis. p53 and its downstream effector p21 were activated concomitantly to the growth arrest with a recovery of the basal levels at 24 h. Moreover, MAP kinase ERK1/2 that is involved in cell cycle progression, was inhibited during the growth arrest period. ERK1/2 re-activation was essential to protect cells from apoptosis, as by the use of a specific inhibitor we were able to block the re-entry in G1 and to trigger apoptosis in AGS cells. We suggested that, in AGS cells, the pro-oxidant effects of DADS were efficiently counteracted by the observed increased concentration of glutathione and activities of glutathione-associated enzymes. Finally, we demonstrated that by further increasing the expression level of selenium-dependent glutathione peroxidase, cell cycle arrest was totally prevented confirming a pivotal role of this enzyme in counteracting DADS-mediated oxidative stress.

Up-regulation of advanced-glycated product receptors in the brain of diabetic rats is prevented by antioxidant treatment

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Diabetics have at least twice the risk of stroke and may show performance deficit in a wide range of cognitive domains. The mechanisms underlying this gradually-developing end-organ damage may involve both vascular changes and direct damage to neuronal cells, due to overproduction of superoxide by the respiratory chain and consequent oxidative stress. The study aimed to assess the role of oxidative stress on the aldose reductase-polyol pathway, on AGE (advanced glycated end-product)/AGE-receptor interaction and on down-stream signaling in the hippocampus of streptozotocin rats. Data show that, in diabetic rats, prooxidant compounds levels increase while antioxidant compound levels fall. RAGE and galectin-3 content and polyol flux increase while glyceraldehyde-3-phosphate dehydrogenase activity is impaired. Moreover, NFκB (p65) transcription factor levels and S-100 protein are increased in the hippocampus cytosol, suggesting that oxidative stress triggers the cascade of events that finally leads to neuronal damage. Dehydroepiandrosterone, the most abundant hormonal steroid in the blood, has been reported to possess antioxidant properties. When DHEA was administered to diabetic rats, the improved oxidative imbalance and the marked reduction of AGE-receptors paralleled the reduced activation of NFκB and the reduction of S-100 levels, reinforcing the suggestion that oxidative stress plays a role in diabetes-related neuronal damage.

Copper overload induces oxidative stress and mitochondrial damage in SH-SY5Y human neuroblastoma cells

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Wilson′s disease is an inherited alteration of copper homeostasis, due to mutations in a copper transporting p-type ATPase (ATP7A), which is associated with copper overload in brain, in addition to liver. Studies on copper toxicity in liver cells in experimental models of Wilson′s disease propose the involvement of mitochondrial damage, but little is known about the mechanisms underlying neurodegenerative processes. In the present study, human SH-SY5Y cells were treated with copper sulphate (from 50 to 300 μM), in complete medium, for 24 hours. As a consequence, copper levels increased in whole cells and to a higher extent in isolated mitoplasts, an increment which resembles that one observed in Wilson′s disease brain. Toxic effects of copper resulted in impaired capability of mitochondrial dehydrogenases to reduce a tetrazolium salt, which was an earlier damage than the loss of the integrity of the plasma membrane, effect that seems to be amplified by the simultaneous treatment with ascorbate. Neither the change in the cell cycle profile, nor the activation of caspase-3, nor the chromatin condensation was observed under these experimental conditions, thus ruling out the occurrence of an apoptotic process. The formation of ROS occurred upon copper treatment, while protein levels of the subunits of complex I and of complex V of the respiratory chain, as well as those of pyruvate dehydrogenase decreased. These findings, then, strongly support the hypothesis that neurodegeneration in Wilson′s disease is associated with oxidative stress and mitochondrial alterations.

Acknowledgements: this study was supported by a grant from the Italian Ministry of Health to L. R.

Antioxidative activity of Mn-substituted Bacteriochlorophyll *a* derivatives – A novel class of catalytic antioxidants

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Enzymatic and molecular antioxidants play a critical role in the cellular defense mechanisms against the chemical damage induced by oxygenic and nitrogenous reactive species (ROS and RNS, respectively). Peroxynitrite ($ONOO^-$) is a highly reactive and toxic molecule that is generated in the cell *via* a high-rate reaction between superoxide ($O_2^{\cdot-}$) and nitric oxide (NO) radicals. The toxic effects of $ONOO^-$ have been attributed to various pathologies such as Alzheimer's disease, rheumatoid arthritis, atherosclerosis, lung injury, and other diseases. Hence, antioxidants that can scavenge $O_2^{\cdot-}$ or NO could inhibit the formation of peroxynitrite and prevent its induced oxidative stress.

Recently, we have examined the antioxidative capabilities of a novel Mn substituted Bacteriophoephorbide compound, ([Mn]-Bphied, where Bphied is a synthetic derivative of the bacterial photosynthetic pigment, Bacteriochlorophyll *a*). Our studies show that the compound can scavenge both $O_2^{\cdot-}$ and NO. Antioxidative action towards $O_2^{\cdot-}$ was demonstrated by inhibiting $O_2^{\cdot-}$-induced death of endothelial H5V cell cultures. The extent of cell survival was found to be correlated to the increase of [Mn]-Bphied concentration. Antioxidative action towards NO-induced damage was demonstrated in wild-type *Arabidopsis italiana* leaves that were exposed to excess amounts of NO. The NO treatment resulted in severe chlorophyll degradation. Introduction of [Mn]-Bphied to the leaves either by injection or infiltration completely inhibited the chlorophyll degradation. Our results lay the foundations for the development of the [Mn]-Bphied system as a novel class of catalytic antioxidants, which might be used in the research and treatment of oxidative stress-related pathologies.

A new biologically active caffeic acid-derived polymer from *Symphytum asperum* and *S. caucasicum*

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Four high-molecular (>1000 kDa) water-soluble preparations were isolated by ultrafiltration of crude polysaccharides from the Symphytum asperum and S. caucasicum roots and stems. According to IR, 1H, 13C NMR, APT, 2D heteronuclear 1H/13C HSQC spectral data and 1D NOE experiment, the main component of preparations is one and the same new regular caffeic acid-derived polymer, namely, poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene] [1,2]. Such phenolic biopolymer, to our knowledge, is hitherto not known and has been identified for the first time. This compound represents a new class of natural polyethers with a residue of 3-(3,4-dihydroxyphenyl)glyceric as the repeating unit. These Symphytum preparations displayed strong antioxidant activity in DPPH assay and a cell-free hypoxantine/xantine-oxidase system, inhibited lipid peroxidation in phospholipid liposomes, and strongly decreased superoxide anion generation in either the reaction of phenazine methosulfate with NADH and molecular oxygen or in rat PMA-activated leokocytes [3]. Besides, they exhibited high anticomplementary activity and the ability to inhibit the TNF-α production. The above-mentioned activities point to the Symphytum new polymer as a potent anti-inflammatory, vasoprotective and wound-healing agent.

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Towards a better understanding of the mechanism involved and biological relevance in HPLC-DPPH and HPLC-ABTS methods for assessing antioxidants activity

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Antioxidant (AO) activity will differ depending on AO structure and the reactive oxygen species (ROS) involved. The importance of various AO in-vivo depends on which ROS is generated, how and where it is generated, and what the target of oxidation is. In-vitro model systems to investigate AO activity against the various ROS can be a valuable tool in classifying antioxidants. Evaluating existing assays, as to the mechanisms involved and accuracy in predicting AO behaviour, is an important step in this respect. We have selected two widely used on-line methods, targeted at radical chain breaking antioxidants for this purpose: DPPH (2,2'-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) decolouration assays. We have investigated the mechanism and thermodynamics of these methods. We show how data obtained using the assays correlate with in-vivo data. Contrary to previous studies [1], we propose that the results provided by these on-line assays are governed by the thermodynamics rather than the kinetics of the reactions taking place. Thermodynamic models were applied to provide a better understanding of the characteristics assessed by these assays. Equilibrium constants extracted from the data are compared with AO oxidation potentials given in literature.

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Improved FIA-ABTS assay for measuring total antioxidant activity (TAA) of Verdicchio wines (VW), extra virgin olive oil (EVOO), and fruits

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TAA considers the cumulative action as well as the synergistic interaction of all the antioxidants present in food giving its capacity to scavenge free radicals. TAA is useful for screening fruits, vegetables and their by-products (e.g., olive oils and wines), which antioxidant contents depend on both cultivar varieties and manufacturing processes. Assays were performed with an improved FIA-ABTS method where both temperature and dispersion of sample and reagent were strictly controlled. TAA hierarchy in fruit was: wild strawberries >> cultivated strawberries >> kiwifruit = apples = apricots = peaches. Differences were found in apricots and peaches grafted from different rootstocks. A preliminary genetic study on strawberry varieties and advanced selections investigating their quality and nutritional value was carried out. Differences of quality and nutritional parameters were found within strawberry species and among genotypes of cultivated strawberry. VW revealed average TAA accompanied by consistent polyphenol contents. EVOOs (native cultivars Raggia/Raggiola) displayed higher TAA than commercially available oils. Results suggest that a boost to local cultivars should improve the development of products that are irreplaceable components of the Mediterranean Diet and improving knowledge on fruit features of a larger genetic base is essential to better define a breeding programme finalized to modify fruit antioxidant patterns and contents.
Studies supported by ASSIVIP, AMPO and COST Action 863.

Validation and application of a lipophilic assay for the measurement of human plasma antioxidant capacity: The Total Antioxidant Performance (TAP)

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The antioxidant capacity of human plasma was determined following the oxidation kinetics of the lipid soluble BODIPY 581/591 fluorescent marker using MeO-AMVN as lipophilic radical initiator. The results were expressed as percentage of BODIPY 581/591 green fluorescence inhibition (TAP value) with respect to control (phosphatidylcholine). The suitability of the assay was evaluated on the basis of its precision, reproducibility and specificity. The intra- and inter-assay CV% were both under 5%. The addition of phosphatidylcholine (up to 750 μg/ml), a representative substrate of plasma peroxidation, did not induce significant changes in the TAP value. Also, no BODIPY 581/591 photo-oxidation was observed during the experimental time-course (220 min). The TAP values of 6 plasma samples from healthy donors were measured and correlated with the main plasma antioxidants (uric and ascorbic acids, α-tocopherol, carotenoids) and lipid profiles. Significant correlations were found between TAP and UA ($r = 0.97$, $p < 0.05$) and cholesterol-adjusted α-tocopherol ($r = 0.934$, $p < 0.01$). The results confirmed that the TAP assay is suitable to measure the antioxidant activity of plasma antioxidants localized in both the lipophilic and hydrophilic compartments. (Supported by USDA 1950-51000-065-02A)

Alterations in the erythrocyte glutathione system in mild cognitive impairment: Similarities with Alzheimer's disease

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Introduction: Oxidative stress is one of the factor involved in the pathogenesis of Alzheimer's disease. It means an imbalance between the formation and spread of reactive oxygen species and the antioxidant defences. Specific antioxidant enzymes have a crucial role in the prevention of these deleterious effects. The aim of this study was to determine the effects of aging and dementia of the Alzheimer type (AD) on glutathione system in erythrocytes. *Methods*: The present study was carried out in patients with mild cognitive impairment (MCI), patients with AD. Both groups of patients were compared to healthy elderly ones without signs of dementia (control group). Erythrocyte glutathione peroxidase (GPx), glutathione reductase (GR) activities and the levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were measured in three different groups of patients. *Results*: The results showed that GR activity in the erythrocytes of elderly-aged with MCI patients and in patients with a diagnosis of AD decreased versus control group. However, the levels of GPx activity, GSH levels, and ratio of GSH/GSSG in the erythrocytes of the patients with MCI and AD were significantly decreased compared to control subjects. *Conclusion*: The present results show that oxidative damage appears as one of the earliest pathophysiological events in AD, and patients with MCI and AD have similar regulation of circulating oxidative stress markers. These changes may be possibly related to the progression of the disease. The increased activities of erythrocyte antioxidant enzymes may be a compensatory upregulation in response to the increased oxidative stress.

Mechanistic studies of the reaction of peroxynitrite with nitrosyliron(II)myoglobin and nitrosyliron(II)hemoglobin

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Nitrosyliron(II)hemoglobin ($HbFe^{II}NO$) and nitrosyliron(II)myoglobin ($MbFe^{II}NO$) have been proposed to represent a stabilized form of $NO^{\bullet}$. The dissociation of $NO^{\bullet}$ from these complexes is very slow (10^{-5}–10^{-4} s^{-1}), whereas it occurs at a rate of 1–10 s^{-1} from the oxidized forms, $HbFe^{III}NO$ and $MbFe^{III}NO$. Therefore, it is conceivable that the release of $NO^{\bullet}$ may first require the outer-sphere oxidation of the iron center:

(1) $HbFe^{II}NO + Oxidant \rightarrow HbFe^{II}NO$

(2) $HbFe^{III}NO \rightarrow HbFe^{III} + NO^{\bullet}$

Among other oxidants, it has been shown that peroxynitrite ($ONOOH/ONOO^-$) can oxidize $HbFe^{II}NO$ [1]. Preliminary results show that a similar reaction takes place with $MbFe^{II}NO$, both in the absence and in the presence of carbon dioxide. Peroxynitrite is known to react with carbon dioxide to produce 1-carboxylato-2-nitrosodioxidane ($ONOOCO_2^-$). This adduct partly decays to $CO_3^{\bullet-}$ and $NO_2^{\bullet}$. Therefore, to better understand the mechanism of the reactions of peroxynitrite with $HbFe^{II}NO$ and with $MbFe^{II}NO$ in the presence of carbon dioxide, we also carried out a pulse radiolysis study of the reactions of $HbFe^{II}NO$ or $MbFe^{II}NO$ with $CO_3^{\bullet-}$. Preliminary experiments show that also these reactions proceed in two steps: oxidation of the iron center to yield $HbFe^{III}NO$ or $MbFe^{III}NO$ and subsequent dissociation of $NO^{\bullet}$. Possible mechanistic hypotheses will be discussed.

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The influence of manganese on chose metal concentrations in mice tissues

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Manganese plays an essential role in antioxidant defences being a part of antioxidant enzymes (superoxide dismutase, glutamine synthetase, alkaline phosphatase and arginase). At low concentrations manganese can have protective effect against hydroxyl radicals. Manganese excess in organism can disturb metabolism of other elements and limit they availability. The aim of our experiment was to estimate influence of manganese chloride on the concentrations of manganese, selenium, copper, zinc and iron in mice tissues. Experiment was carried out for six weeks on male Albino Swiss mice into 4 groups, 20 mice each. The I group was the control group and the mice received redistilled water to drink, while the other groups received manganese chloride ($MnCl_2$) in concentrations (counted on metal): group II – 10 $mg.dm^{-3}$, group III – 20 $mg.dm^{-3}$, group IV – 40 $mg.dm^{-3}$. Manganese, copper, zinc, iron and selenium concentrations were measured in liver, kidneys and brain by ICP-OES method. Manganese chloride administration induced increase of all metals concentrations in examined tissues of mice. Increase of those metals content was dependent on manganese doses. Concentrations of Mn, Zn, Fe and Cu were statistically significant higher in kidneys only at the highest dose of $MnCl_2$ (40 $mg.dm^{-3}$) and brain (20 $mg.dm^{-3}$ and 40 $mg.dm^{-3}$) while in the case of Se only slihtly increases were observed. In liver content of Mn, Zn, Fe and Se only at the highest dose while Cu content was only slightly increased.

Antioxidant versus anticancer properties of hydroxycinnamic acid derivatives

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Trihydroxycinnamic acid and its ethyl ester derivatives were synthesised and evaluated for their antioxidant and anticancer activities. The ester derivatives exhibit a higher radical scavenging activity, when liposomes were used as target systems, a fact which may be related to their conformational preferences and their lipophilicity. The compounds were found to display significant growth-inhibition and cytotoxic effects towards a human cervix adenocarcinoma cell line (HeLa). The partition coefficients (logP) were found to correlate well both with their structural characteristics and with their antioxidant/anticancer activities. Further research is on progress to find leaders that possess both properties, which may be valuable agents for cancer therapy.

Acknowledgements Christophe Siquet acknowledges FCT (SFRH/BD/ 7005/ 2001).

Red wine extends lifespan in *Drosophila melanogaster*

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The beneficial properties of red wine were recognised many years ago, when Greek doctor Galeno prescribed grapes to his patients. The French Paradox relates moderate red wine consumption with a longer lifespan. Red wine is rich in many substances such as polyphenols, whose antioxidant properties are well documented. So, we studied the effects of red wine consumption on life span and gene expression in a controlled population of Drosophila melanogaster. We also performed some studies in Wistar rats to corroborate our results in mammals. For this purpose, we divided flies into three groups: control (C), red wine (RW), non-alcoholic red wine (NRW). Our results showed and increase in mean lifespan in RW flies and even more pronounced in NRW (19%) compared to C flies. In order check whether this effect had a molecular explanation we measured catalase gene expression (an antioxidant enzyme) and a marker of aging, such as 16S mRNA subunit. We found the RW and NRW rats over-express both markers. To check if these beneficial effects could be extrapolated to mammals we determined catalase gene expression and activity in brain of rats which drank water, red wine or non-alcoholic red wine. We obtained that the expression of such enzyme in RW or NRW rats were increased compared to C rats. In conclusion, red wine and even more non-alcoholic red wine extends lifespan in Drosophila melanogaster at least in part by modifying longevity-related gene expression.

Resveratrol prevents peroxynitrite-induced endothelial cell death through mitochondrial pathway

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Atherosclerosis is a chronic inflammatory condition associated with oxidative stress partly due to an increase in the production of oxidant species, namely peroxynitrite. Resveratrol (Res.) is a natural phytoalexin found in grapes and believed to be one of the major constituents of red wine responsible for its cardioprotective effect. The aim of this study was to identify the signalling pathways by which peroxynitrite induces bovine aortic endothelial cell death and to examine whether resveratrol prevents $ONOO^-$-mediated cell death. Peroxynitrite (400-600 μM) is cytotoxic to BAEC triggering a programmed cell death pathway, as indicated by the increase of nuclei condensation and fragmentation, after Hoescht nuclei staining. To test the putative protection of Res, several concentrations at different pre-incubation times were used. Our results show that a longer pretreatment of cells with Res. is more effective in preventing $ONOO^-$-mediated cell death than a shorter one, an effect that is concentration-dependent. Caspases activation analysis by cleavage of fluorogenic substrates, shows that the molecular pathway leading to ONOO- cytotoxicity is associated with an activation of caspase-3,-8 and -9, suggesting that both mitochondrial and death receptors apoptotic pathways are involved. Pretreatment of cells for 14h with 50 μM of Res. efficiently prevents the activation of caspase-9 and-3, but not the activation of caspase-8. In conclusion, our results suggest that resveratrol may contribute to the proposed cardioprotective effects of red wine by interrupting the mitochondrial pathway involved in $ONOO^-$-induced cell death.

Paula Brito is a recipient of the grant SFRH/BD/7986/2001. Supported by POCTI 735019/AGR72000 and POCTI/AGR/599919/2004.

The double blind study of effectiveness of the biological active addiction "selenium-active" in a complex treatment of coronary heart disease

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Objective: To study the influence of food biologically active addition "Selenium-active"(SA) manufacture by "Diod" Ltd. on haemodynamics, biochemical parameters and tolerance to physical activity at patients (PTS) with high blood pressure (HBP), dyslipidaemia (DL), and coronary heart disease (CHD). Material and methods: 60 PTS with HBP, DL and CHD with the positive veloergometry test (VT) in spite of standard antianginal drug therapy. PTS were randomized on 2 groups: reception SA of 2 tablets in day or placebo within 3 months. SA contains vitamin C of 50 mg and selenium of 50 μg. Research was spent by a double blind method. Defined a level of the general, LDL and HDL cholesterol. The general antioxidative index (AI) was counted. Results. In group of the PTS received SA, significant gain of carried out loading, on the average on 2,0 ± 0,5 ($p < 0,0001$) in comparison with placebo where this parameter has changed slightly. At SA PTS in 3 months of reception ischemic depression of segment ST on peak of loading in comparison with results of initial VT (16 vs 21 p., $p < 0,01$) authentically less often was observed. Similar changes were observed at the analysis of frequency of occurrence angina attacks during loading (15 vs 23 pts, $p < 0,01$). At the SA PTS but not placebo decreases of both SBP/DBP levels (-7,2 ± 2,5 and -5,0 ± 1,4 mmHg; $p < 0,01$; $p < 0,001$) was observed. There were decreases in common and LDL cholesterol levels (-9,4% and -13,8 %, accordingly) in SA PTS only. The rate of AI gain in SA PTS has exceeded rate of AI gain in placebo PTS on 14,9%. Conclusion: The biologically active addition SA has a positive influence on homodynamic and biochemical parameters in CHD PTS.

Do nitroxides induce oxidative stress in erythrocytes?

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Nitroxides can prove a superoxide dismutase-mimic activity. They react with superoxide anion ($O_2^{\bullet -}$), forming oxidized, intermediate form, oxo-ammonium cation, which is rapidly reduced by another $O_2^{\bullet -}$. Nitroxides can also induce catalase-like activity of hemoglobin and myoglobin. Moreover, nitroxides can prevent oxidative damage by oxidizing reduced metal ions (ferrous and cuprous). Many recent works have informed that nitroxides have protective effect on oxidative damage in various biological systems.

These studies were undertaken to check whether nitroxides can induce oxidative damage in human red blood cells. The effect of five different nitroxides (piperidine, pyrroline and pyrrolidine derivatives) on lipid and protein oxidation in RBC was examinated. The extent of oxidative damage in erythrocyte were determined in the presence of increasing concentrations of following nitroxides: Tempol, Tempamine and pyrrolin, pyrrolidin derivatives. Additionally, the activity of catalase was estimated. No statistically significant changes in catalase activity was observed. The investigated nitroxides did not increased lipid peroxidation either. The degree of oxidative modification of RBC proteins was measured by monitoring the carbonyl group formation. A small increase in the amount of carbonyl groups was found at lower nitroxides concentrations. Higher nitroxide concentrations caused a decrease in protein oxidation to the level of control. The observed changes were, however, not statistically significant.

In conclusion, we did not found any significant changes in the level of lipid and protein oxidation in human red blood cells.

Ferulic acid derivatives: A structural and biological study

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Ferulic acid (FA) and its derivatives (e.g. alkyl esters) have been the subject of several studies due to their antioxidant and radical scavenging activities. In order to get a better insight into the biological action of this kind of phenolic compounds, the following study was performed: i) conformational analysis by vibrational spectroscopy and theoretical methods; ii) calculation of the BDE and IP values and correlation with the experimental antioxidant activity; iii) evaluation of the cytotoxic activity in human cancer cell lines; iv) evaluation of effect of FA on the thermal denaturation of calf thymus DNA in solution, by micro-DSC and UV-Vis spectroscopy.

Acknowledgements R. Calheiros is grateful to FCT for a PhD fellowship (SFRH/BD/16520/2004).

Biological effects of human serum red wine metabolites on endothelial cell transcription factor activation and gene expression

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There is evidence that moderate red wine consumption may be protective against the development of coronary artery disease (CAD), by decreasing inflammatory activity and improving impaired endothelial function. The aim of this study was to determine the effect of red wine on pro-inflammatory cytokine-mediated endothelial cell activation, taking into consideration polyphenol modifications that occur during gastro-intestinal absorption and alcohol. For this purpose, we set up an experimental model using a limited number of healthy subjects as bioreactor. Serum isolated before (control serum) and after (RW serum) red wine supplementation (5ml/kg), was used to enrich the culture medium of primary human endothelial cells before TNF treatment. Preliminary results on the kinetic of TNF-mediated transcription factor activation show that RW serum pre-incubation delays NF-κB and AP-1 nuclear translocation with respect to control. We also analyzed the molecular expression of 600 specific genes involved in cardiovascular disease using Atlasä human cardiovascular array. Endothelial cell incubation with RW serum was associated with down-regulation of several genes involved in coagulation and fibrinolytic process. In other hand, RW serum and TNF treatment regulated several genes involved in the coagulation pathway in a pro-coagulant manner. In spite of the large number of studies addressing the effects of polyphenols on TNF mediated endothelial cells modification, the real effects of red wine metabolites has never been investigated.

This research was supported by the Italian Ministry of Agriculture and Forest Policy (MiPAF), “Food Quality” Project

Inhibition of lung tumour cell proliferation by ω-3 and ω-6 polyunsaturated fatty acids: Molecular mechanisms

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Altered levels of polyunsaturated fatty acids (PUFA) or their metabolites are involved in the onset of some pathologies, including cancer. The quantity and quality of dietary fat intake is correlated with the incidence of several tumour types. PUFAs can differently affect tumour growth, even if reported results are contradictory: in general, ω-3 PUFAs inhibit tumour growth, whereas ω-6 favour it. In this study human lung tumour A549 cells have been exposed to arachidonic (AA) or docosaexaenoic (DHA) acids to investigate the effect of both ω-6 and ω-3 PUFAs on proliferation, and to elucidate the mechanisms involved. Both fatty acids block the proliferation and the effect is mainly mediated by the induction of lipid peroxidation, as evidenced by the preventive effect of antioxidants. The signalling transduction pathway leading to growth arrest involves decreased DNA-binding activity of AP-1. In AA-treated cells, growth inhibition is also partially due to the modulation of COX-mediated eicosanoid production: in fact, indomethacin, a COX inhibitor, partially prevent the effect of AA, not that of DHA. Differently, LOX inhibition has no effect. In conclusion, both ω-3 and ω-6 are able to block tumour cell growth, even if some differences in signalling pathways involved have been evidenced, in consequence of the different properties (pro-or anti-inflammatory) of eicosanoids derived from AA and DHA.

Research supported by Compagnia San Paolo, Regione Piemonte, University of Turin

Blockade of JAK2 with AG490 protects endothelial cells against oxidative stress

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Increment of endothelial cell (EC) survival is a principal aim in present cardiovascular therapy. Recently, an implication of the Jak/Stat pathway in protection against reactive oxygen species (ROS)-induced damage has been reported in astrocytes and smooth muscle cells. The aim of the present study was to examine AG490-induced anti ROS protection in other relevant cell types. EC from human umbilical vein (HUVEC) and bovine aorta (BAEC) and pig tubular proximal epithelial cells (TC) were incubated (48h) with different concentrations of H_2O_2 or hypoxanthine/xanthine oxidase (HX/XO), in the presence of the Jak2 specific inhibitor AG490 (50 μM) or vehicle. Methods included cell damage measurement (LDH release), Western blot for Bcl-2 and Bax, and HIF1α analysis in nuclear and cytosolic extracts. Caspase 3 activity was assayed by the fluorogenic substrate Ac-DEVD-AMC. Jak/Stat pathway inhibition with AG490 has strong protective effects on both EC and TC against oxidative stress induced by H_2O_2 or HX/XO, at high cytotoxic concentrations (250 μM-1 mM H_2O_2; 12.5 mU XO). In these conditions, AG490 induced changes in at least 3 mechanisms with possible protective effect: 1) Increased nuclear binding of HIF1α. 2) Increased levels of the antiapoptotic protein Bcl-2; 3) Reduced caspase 3 activity. *Conclusions*: 1. Jak/Stat pathway inhibition protects EC from oxidative stress. 2. This type of protection involves induction of HIF1α nuclear binding, increased Bcl-2 levels and reduced caspase 3 activity.

Redox regulation of cell signaling during early stages of epithelial carcinogenesis

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In our study, a model of human epithelial carcinogenesis was established. Human gingival mucosal keratinocytes (GM16) were immortalized with oncoproteins E6 and E7 of human papillomavirus type 16. This did not cause these cells to be transformed. Stress from chronic ethanol treatment was able to produce keratinocyte cell clones. Upon subcultures, we produced two cell populations classified according to their morphology: epithelium-like (EPI) cells, and fibroblast-like (FIB) cells (Eur J Cell Biol 82, 1, 2003). FIB cells were more transformed than EPI cells because they grew anchorage independently and exhibited greater motility. We were interested in redox status of these cell lines, in particular, the source of superoxide radical gp91phox homolog Nox1, which had been previously described to cause tumorigenic conversion of mouse fibroblasts. FIB cells expressed Nox1 mRNA and protein more than EPI cells. Concomitant increases of ERK1, JNK1, and phospho-JNK2 proteins were observed in FIB cells (Oncogene 22, 6045, 2003). FIB cells also expressed increased inducible nitric oxide synthase on the mRNA and protein levels. iNOS was found to be downstream of MAP kinase pathways (Nitric Oxide 11, 237, 2004). Recent experiments indicate that FIB cells progress even further into a more transformed phenotype capable of proliferating in an absence of serum, exhibiting greater invasiveness and forming branched-like morphology on Matrigel. Furthermore, Nox1 overexpression renders GM16 cells resistant against Ca+2/serum-induced differentiation. Taken together, our data demonstrate increased redox-dependent gene expression and activities in cells exhibiting a more transformed phenotype, and that Nox1 plays a role in signaling during transformation of human epithelial cells.

Protective effects of cyanidin-3-glucoside against UVB-induced response in human keratinocytes

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One of the most significant risk factors associated with the development of skin disease is exposure to UV radiation from the sun. UVB light activates different pathways leading to skin aging and eventually to skin cancer. Anthocyanins, a group of flavonoids present in many common vegetable foods, are under investigation for their chemopreventive activity. The aim of this study was to evaluate the efficacy of cyanidin-3- O-glucoside (C3G) on modulation of cellular responses to UVB exposure in human keratinocytes (HaCaT) and particularly on both inflammation and apoptosis pathways. Our results indicate that UVB radiation affects cellular integrity of HaCaT cells only at a UVB dose >20 mJ/cm^2. The translocation of pro-inflammatory transcription factors NF-κB and AP-1 increases 1 hour after UVB exposure while lower activation levels were detected in C3G- treated cells. To evaluate the entity of inflammation we assessed IL-8 mRNA. High mRNA levels of this proinflammatory cytokine are detected upon UVB exposure and a significant decrease is associated with C3G pre-treatment. Our results show that UVB also activates procaspase-3 cleavage, an executioner key in apoptotic signalling pathway; at the same UVB dose, C3G inhibits caspase-3 activation with values similar to sham-irradiated controls. Inhibition of apoptosis is confirmed also by markedly decreased DNA fragmentation in C3G treated cells exposed to UVB dose of 20 mJ/cm^2. Taken together, our data show that C3G protects against the adverse effects of UVB radiation and provides a molecular basis for the photochemopreventive effects of C3G.

Investigating the use of metal chelators to identify transition metals in PM: A novel approach

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Adverse health effects attributable to ambient particulate matter (PM) are a major concern worldwide. Studies have shown that the transition metal content may be at least partly responsible for its toxic effects. PM presents a particular analytical challenge as metals are often present at very low concentrations (nM) within a complex matrix. Therefore, methods to determine metal in PM must exhibit high degrees of sensitivity and selectivity. Techniques such proton-induced x-ray emission spectroscopy (PIXE) can measure total concentrations of metals and other elements, but for toxicological purposes the soluble (bioavailable) fraction would yield more useful data. In solution, transition metals are able to oxidise molecules such as ascorbate (AA). We can modify the degree of AA loss by using metal chelators to selectively separate, mobilise or inactivate redox-active transition metals; thus forming the basis of a simple bio-assay to distinguish these metals in PM.

AA concentration (μM ± SD) after 2 hr incubation (n = 6)

	Fe	Cu	V
Metal Only	173.5 (17.7)	0	187.0 (9.2)
+ EDTA	0	181.8 (23.5)	174.7 (10.3)
+ DFO	192.2 (5.8)	180.0 (12.8)	66.6 (13.5)
+CP20	151.8 (2.1)	30.0 (10.0)	195.5 (3.4)

200 μM AA was incubated with 1μM $FeCl_3$, $CuSO_4$, $CoSO_4$, $MnCl_2$, $NiSO_4$ or $VOSO_4$ (pH 7.5), as well as t_0 and t_{2hour} metal free controls, at 37°C for 2h. AA concentration was then determined by HPLC with electrochemical detection. We also added chelators such as EDTA, DFO, CP20 to the metals (10 μM) and observed the effect on ascorbate depletion compared to metal alone. So far we are able to distinguish between Cu, Fe and V using a combination of CP20, EDTA and DFO (see table). Further experiments will determine whether this method can be used successfully with PM samples.

An innovative technology for improving solubility and antioxidant properties of coenzyme Q10

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CoQ10 is a powerful antioxidant and an important micronutrient for better health.The main disadvantage of Q10 is the poor solubility which leads to bad bioavailability.Therefore it is a challenge for scientists and for the pharmaceutical and food supplement industry to improve the bioavailability for better products and for a better benefit for the patients. CoQ10 is absorbed slowly; peak plasma levels are attained within 5–10 hours following oral administration. Coenzyme Q10 absorbtion problems are mainly related to its scarce solubility in physiological environment. Well over 60% of an oral dose is excreted in the feces. Actimex has developed and patented a solid state technology (mechanochemical activation) which leads to the production of new multicomposite systems, where the active compound is brought to an optimized (activated) physico-chemical state that allows improvement of the biopharmaceutical properties of the native compounds without altering their chemical structure. The versatility of this technology has allowed its application also to CoenzymeQ, despite its low melting point ans wax-like appearance, leading to a multicomposite material where solubility of CoQ10 has improved more than 50 times over the native compounds. Moreover, an in-vitro test (ORAC-Oxigen Radical Absorption Capacity) has been set up to verify the anti-oxidant properties of the multicomposite vs the native compound; an increase of antioxidant properties (at equivalent CoQ10 concentration) has been observed for activated CoQ10 in the multicomposite vs native substance.

Absorption and metabolism of olive oil simple phenols in the small intestine

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Olive oil polyphenols contain phenolic acids, mainly hydroxytyrosol (HT) and tyrosol (TYR), and their conjugate forms, that have been demonstrated to exert many beneficial biological effects. However, the bioactivity of these compounds in vivo depend on the extent of their absorption and metabolism. We investigated the transport and metabolism of olive oil polyphenols across the small intestine using the human caco-2 monolayer and isolated preparations of rat jejunum and ileum. Apparent permeability coefficients (Papp) were found to be always significantly higher than $1x10^{-6}$, indicating that HT and TYR are very well absorbed in humans. Major metabolites identified were O-methylated form for HT, glucuronides for HT and TYR and glutathionyl conjugate for HT. Although gastrointestinal metabolism decrease HT and TYR bioavailability, their metabolites could also be capable of exerting biological effects, as has been shown for many other plant derived polyphenol metabolites.

Supported by PIC-Interreg III

Protein S-cysteyl-glycylation: a novel function of membrane gamma-glutamyltransferase on cellular and extracellular proteins

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Previous studies have documented that activity of the plasma membrane enzyme γ-glutamyltransferase (GGT) is accompanied by prooxidant processes, with production of reactive oxygen species and oxidation of cellular protein thiols. The present work was aimed to verify the occurrence and extent of S-thiolation mediated by GGT and characterize the molecular species involved in mixed disulfide formation. Experiments show that the cysteinyl-glycine (CG) originating from cellular GGT-mediated GSH metabolism can efficiently thiolate cellular proteins, as well as proteins present in the extracellular environment. With cells presenting high levels of GGT expression, basal levels of CG-containing protein mixed disulfides are detectable, in cellular proteins as well as in proteins of culture medium. Stimulation of GGT activity in these cells by administration of substrates results in an increase of CG mixed disulfide formation and a concomitant decrease of GSH-containing disulfides, likely due to GGT-dependent removal of GSH from the system. The findings reported suggest that binding of CG ("protein S-cysteylglycylation") may represent an as yet unrecognized function of membrane γ-glutamyltransferase, likely playing a regulatory role(s) in the cell and its surroundings.

The influence of pyrrolidine nitroxides on toxicity of doxorubicin and hydrogen peroxide

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Nitroxides, an effective antioxidants, have been proposed as compounds able to attenuate the cardiotoxic effect of anticancer drugs e.g. doxorubicin. Biological properties of nitroxides depend on many factors such as their permeability and the rate of reduction in cells. Of great importance is the ring structure and ring substituents of the nitroxide. Thus we investigated the biological properties of two pyrrolidine nitroxide derivatives 2,2,5,5-tetramethyl-3-carbamoylpyrrolidin-1-oxyl (PD) and 2,2,5,5-tetramethyl-3-carboxypyrrolidin -1-oxyl (CPD), which differ in ring substitution in position 3 of the pyrrolidine ring and their effect on toxicity of anthracycline anticancer drug – doxorubicin (DOX). For comparison we estimated the ability of these nitroxides to protect cells against H_2O_2, which is generated in cells during the redox cycle of doxorubicin. Neither compound revealed any significant cytotoxicity up to milimolar concentrations. Pretreatment of immortalized hamster peritoneal cells (B14 line) with CPD before their incubation with DOX resulted in increased cell survival, which suggests the protective effect of this compound against toxicity of DOX. Under the same conditions minor effect of PD was found. The nitroxides did not show significant influence on cytototoxicity induced by IC_{50} concentration of H_2O_2 at concentrations 10-500 _M. These results suggest that the substituents of the heterocyclic ring are important and decisive for the antioxidant properties of nitroxides.

Functional studies on carbonylation produced by acrolein on actin

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Cys thiol groups in proteins react readily with acrolein, as do nucleophilic N atoms in Lys and His. During the reaction of acrolein with proteins, Michael reactions involving nucleophilic addition to the unsaturated β-carbon of acrolein predominate over Schiff-base reactions at the aldehyde group. As Michael adducts retain an intact carbonyl group, marked carbonylation occurs. In Alzheimer's disease brain, increased protein-bound acrolein adducts were detected and target proteins, including actin, have increased carbonyl groups. Further, disruption of actin filaments was shown in human gingival fibroblasts exposed to acrolein, one of the volatile fractions of cigarette smoke. We exposed isolated actin to acrolein (1:1, 1:10, 1:20, and 1:50 mol/mol) for 1 h. Acrolein-induced carbonyl groups were evidenced by Western-blot probed with anti-2,4-dinitrophenyl-idrazone antibodies. Actin polymerization, triggered by physiological salt concentration, was followed fluorometrically by the fluorescence intensity increase of trace quantities of pyrenyl-actin added to acrolein-treated actin. Acrolein adduction resulted to inhibit actin polymerization, both as extent and rate, in a dose-dependent manner. The site(s) of acrolein adduction were then identified by a mass spectrometric approach (see abstract presented by Aldini and colleagues).

Mechanisms of bleomycin-mediated signal transduction

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Bleomycin is an oxidative stress-inducing agent, which can cause pulmonary fibrosis. Signal transduction mechanisms of bleomycin, however, have not been well defined. We have shown that belomycin activates signal transduction and transcriptional regulatory mechanisms for the expression of angiotensin-converting enzyme (ACE) and gamma-glutamylcysteine synthetase (γ-GCS). Treatment of primary bovine pulmonary artery endothelial cells with belomycin increased ACE protein and mRNA expression and activated 97-bp ACE promoter. Belomycin activated p42/p44 MAP kinase (ERK) and nuclear translocation of Egr-1, a regulator of ACE gene transcription. Bleomycin-induced activation of ERK and Egr-1 was inhibited by a MEK inhibitor, indicating the activation of MEK/ERK/Egr-1 pathway by bleomycin. We also found that MEK/ERK pathway is involved in the bleomycin-induced activation of NF-κB which regulates γ-GCS expression. γ-GCS promoter is also regulated by anti-oxidant response element (ARE), and our results indicated that bleomycin activates the binding of Nrf-1 and –2 to ARE. N-acetylcysteine inhibited belomycin-induced Nrf activation, but not NF-κB activation. These results suggest that bleomycin activates redox sensitive pathway for the activation of Nrf-1 and –2, and redox independent pathway to activate NF-kB, which in turn regulate gene expression of ACE and γ-GCS. These signal transduction pathways may play roles in belomycin-induced pulmonary fibrosis.

Supported by HL73929

Antioxidant status and oxidative stress in intestinal failure long-term home parental nutrition patients

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Aim: We aim to evaluate the relationship between plasma malondialdeyde (P-MDA)and 4-hydroxynonenale (P-HNE) as indexes of oxidative stress with some HPN-related factors and blood antioxidants in Home Parenteral Nutrition (HPN) pts. Methods 41 pts(M:16;F:25), mean age 61.05 ± 14.4 were studied. HPN median duration: 2636 d(172-5499). HPN regimen: n infusions/wk: 5.7 ± 1.15; VitE/PUFA: 0.52 ± 0.32 mg/g/d. Se, vitamins A, C, E were supplemented to maintain blood levels within normal ranges. Univariate analysis with linear regression was performed between both P-MDA and P-HNE and: age, HPN duration, HPN regimen, blood inflammatory indexes(ESR,C-Reactive Protein,WBC), plasma levels of: selenium(P-Se), ascorbic acid(P-AA), dehydroascorbic acid (P-DHA), α-tocoferol, retinol, red blood cell- reduced and oxidized glutathione (RBC-GSH, RBC-GSSG).One way ANOVA was used for sex.The indipendent relation between significant candidates in univariate analysis ($p < .05$) was assessed by a multivariate linear regression(SAS). *Results:* P-MDA (4.7 ± 1.3 UF) is inversely related ($p <. 01$) with Se intake (41.3 ± 20 mg/d). P-HNE (8.1 ± 2.8 UF) is inversely related with Se intake ($p < .02$), plasma-α-tocoferol (8.4 ± 3.8mg/ml) ($p < .05$) and positively with RBC-GSSG (0.11 ± 0.08) ($p < .006$). *Conclusions:* In HPN pts the intakes of Se and vit E currently given to maintain their blood levels whitin normal limit seem inadequate to compensate for oxidative stress and are inversely related to the level of lipoperoxidation.

In vivo pro-oxidant state in Werner syndrome: Results from three patients and two heterozygotes

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The hypothesis was tested that Werner syndrome (WS) phenotype might be associated with an in vivo prooxidant state. A set of redox-related endpoints were measured in three WS patients, two parents, and 99 controls. The following analytes were measured: (a) leukocyte 8-hydroxy-2-deoxyguanosine; (b) blood glutathione, (c) plasma levels of glyoxal, methylglyoxal, 8-isoprostane, and uric acid, ascorbic acid, α- and γ-tocopherol. Leukocyte 8-hydroxy-2-deoxyguanosine levels are significantly increase in the 3WS patients vs. 85 controls. The disulfide glutathione:glutahione ratio was significantly altered in WS patients. Glyoxal and methylglyoxal levels were significantly increased. The plasma levels of uric acid and ascorbic acid also increased significantly in WS patients and in their parents. This is the first report of in vivo alterations of oxidative stress parameters inWS patients.

Effects of different garlic extracts on cell cycle and viability of HepG2 hepatoma cells

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Two major types of preparation of garlic extract, aqueous and oil, are usually used to prepare garlic derivative supplements. In this study we investigated the effect on the cell cycle regulation of HepG2 hepatoma cells, of a fresh aqueous garlic extract prepared with two different kinds of garlic coming from two regions of Italy well known for their garlic production, i.e. Latina (GEL) and Sulmona (GES) The effects of the treatments with GEL and GES were further compared with the oil soluble sulfur compound of garlic, diallyl disulfide (DADS). DADS was not able to induce a significant effect on cell cycle of HepG2 cells, while GEL and GES led to cell arrest in G2/M phase, but to a different degree. The subG1 region (percentage of cell population showing apoptotic features) suggest that the blockage in G2/M phase could result in the following induction of the apoptotic program. According to Western blot analyses, the increase of cells in G2/M phase is supported by an increase of the levels of p53 and its downstream effector p21 (still active in GES 48 hours after treatment). Conversely DADS did not show any significant increase of p53 and p21. We also demonstrated that the apoptosis induction of HepG2 cell by GEL and GES was dependent on the activation of the c-Jun NH2 terminal kinase (JNK)/c-Jun pathway. The immuno-reactive band of p-Jun, the phosphoactivated form of c-Jun, was early evidenced in both treatments, but remained at high level only after 24 hours treatment with GES. The results obtained suggest that the benefits associated with garlic extract consumption is probably due to the whole pool of organosulfur and antioxidant phytochemicals present in garlic, and that garlic grown in different areas do not show the same healthy characteristics.

This work was supported in part by: MIUR, FIRB and Ministero della Salute

VEGF increases HIF-1 levels by a O_2^--mediated mechanism

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The main known angiogenic factor, vascular endothelial growth factor (VEGF) is transcriptionally regulated by hypoxia inducible factor 1 (HIF-1). In normoxia, the HIF-1α subunit is degraded via proteasome and HIF-1 remains inactive. At hypoxic O_2 levels, degradation stops and HIF-1α migrates to the nucleus, where it binds to HIF-1b and then to its specific DNA binding sequences. A number of works has shown that specific cytokines and growth factors activate HIF-1 even in normal O_2 levels. A yet unexplored, putative candidate to regulate HIF-1 is VEGF itself. Endothelial cells (EC) were incubated with VEGF-A_{165}, H_2O_2 or hypoxanthine/xanthine oxidase (HX/XO) for 6 hours. EC were pretreated for 30 min with: MnTMPyP and NAC as O_2^- and H_2O_2 scavenger, respectively, and with apocynin, dicumarol, wortmannin, PD98059, and SB203580, as NADPH oxidase, JNK, Akt/PKB and p42/p44 and p38 MAPK inhibitors, respectively. HIF-1α protein levels were studied by Western blot and confocal microscopy. Both VEGF and HX/XO increase HIF-1_ protein nuclear translocation, in a time/ concentration-dependent manner; H_2O_2 has no effect. The role of O_2^- is reinforced by studies with ROS scavengers and apocynin. Finally, all kinases studied except p42/p44 are implicated in the VEGF- and HX/XO-related HIF-1α increase. *Conclusions*: (1) The VEGF-related increase of HIF-1α is mediated by NADPH oxidases. (2) The O_2^- produced by NADPH oxidases is a necessary signal for HIF-1α increase. (3) This increase is further mediated by JNK, p38 MAPK and PI3K/Akt.

Studies of posible atherogenic effects of phospholipid chlorohydrins

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The oxidation of low-density lipoprotein (LDL) is thought to contribute to atherogenesis. Phagocytes produce a variety of oxidants as part of the innate immune defence, which react both with proteins and lipids, and could contribute to the oxidation of LDL and plaque formation *in vivo*. Myeloperoxidase, an enzyme which is able to produce hypochlorous acid (HOCl), is released from these phagocytes, particularly from neutrophils which are found to occur early in lesion development. HOCl can oxidise lipids to give chlorohydrins. We have formed chlorohydrins from stearoyl-oleoyl phosphatidylcholine (SOPC) – a phospholipid found in LDL – and investigated various biological effects of this oxidation product using both biochemical and physiological assays. SOPC chlorohydrin was found to deplete ATP levels in human myeloid cells, which was both time- and concen- tration-dependent and correlated with a loss of viability using an MTT assay. Furthermore, the chlorohydrin increased leukocyte adhesion to homologous artery segments isolated from atherosclerotic (ApoE knockout) mice, and this was also in a time- and concentration-dependent manner. These are the first data showing effects of a chlorohydrin on leukocyte adhesion and, together with the effects on cell viability, suggest a possible atherogenic / inflammatory role for SOPC chlorohydrin.

A new paradigm for COX-2 signaling: Electrophilic lipids and mitochondrial ROS generation

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It is becoming increasingly recognized that the trans-duction of simple oxidants such as peroxynitrite or hydrogen peroxide to signaling molecules may involve the formation of electrophilic lipid oxidation products. In addition, the endogenous production of biologically active lipids can occur non-enzymatically to yield products such as 4-HNE and the isoprostanes, and enzymatically to yield the prostaglandins and the leukotrienes. However, it is still not clear whether the endogenous activation of enzymes such as cyclooxygenase (COX) can generate electrophilic lipids capable of post-translationally modifying proteins. In addition, the targets for such electrophilic lipids within the cell have not been clearly defined. To examine both these questions, we used tagged arachidonic acid as a substrate for COX and employed confocal microscopy to examine the subcellular distribution of these tagged prostaglandin products. Separation of cell lysates by electrophoresis revealed the formation of stable covalent protein adducts. Furthermore, we compared these results from endogenous products with the *in vitro* addition of purified, single compounds. We have found that compounds containing electrophilic carbon centers co-localize with active mitochondria, and, increase mitochondrial-dependent ROS generation. These results have led us to the hypothesis that COX2-dependent products can control cell function through stimulation of mitochondrial ROS formation.

Oral antioxidant administration impairs mitochondrial biogenesis and antioxidants gene expression in trained rats

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Introduction: Exhaustive exercise generates excessive amounts of free radicals that overwhelm cellular antioxidant defences and may cause tissue damage. They may, however, constitute signals to regulate muscle cell function. This is one of the oldest postulates in the field, dating back to the suggestion that free radicals produced in exercising muscle might stimulate mitochondrial biogenesis and the expression of genes for antioxidant enzymes. Aim: Our aim was to elucidate the role of the free radicals generated in moderate physical exercise, in the expression of antioxidant genes and of transcription factors for mitochondrial biogenesis Material and methods: Twenty male wistar rats were randomly divided into four groups: sedentary controls (n = 5), trained (n=5), trained treated with vitamin C (n = 5) and trained treated with allopurinol (n = 5). Allopurinol acts as an antioxidant because it inhibits xanthine oxidase, an important enzyme in the generation of free radicals in exercise. Where indicated animals were subjected to moderate exercise training five days a week during three weeks. Results: Our results show that moderate exercise up-regulates the expression of antioxidant enzymes associated with longevity, such as Mn-SOD and GPx. We also found that moderate exercise up-regulates the expression of NRF-1 that is a key transcriptional activator of nuclear genes encoding mitochondrial enzymes and Tfam, which stimulates mitochondrial DNA transcription and replication. However, supplementation with vitamin C or allopurinol during training prevents all of these adaptations.

Normal pregnancy affects α- and γ-tocopherol metabolism

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Vitamin E is important in maintaining a healthy pregnancy and depletion may result in foetal morbidity or mortality. Vitamin E status has repeatedly been reported to be elevated in pregnancy. Insight, especially to vitamin E metabolism will provide valuable information on the role of vitamin E during pregnancy. Aim: To assess the effect of pregnancy on the in vivo metabolism of vitamin E (α-T and γ-T). Methods: Plasma α-T and γ-T (HPLC), plasma α-CEHC and γ-CEHC metabolites (GC/MS) and total cholesterol (spectrophotometer) were analysed in 52 healthy pregnant women at 12, 16, 20 and 24 weeks of gestation. For comparison, 22 age-matched non-pregnant women were also examined. Results: At gestation of 12 weeks, both plasma α-T and γ-T were not significantly different to controls. From week 12 to week 24 of gestation α-T and γ-T concentrations significantly increased 40% and 30%, respectively. However, after correction for cholesterol these changes disappear. Both α-CEHC and γ-CEHC were lower at 12 weeks gestation than non-pregnancy (22.3 ± 3.1 nmol/L versus 31.3 ± 2.9 nmol/L and 98.7 ± 11.6 nmol/L versus 116.9 ± 7.2 nmol/L, respectively) and both remained low up until 24 weeks of gestation. Conclusion: The increase of plasma α-T and γ-T during pregnancy is most likely due to elevated plasma cholesterol, and hence its transport capacity. However, the reduced metabolism of α-T and γ-T in normal pregnancy also suggests more efficient retention of α-T and γ-T.

Oxidised palmitoyl-arachidonyl-phosphatidyl choline does not activate Toll-like receptor 1, 2, 4 or 6 signalling

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Oxidised phospholipids (oxPLs) accumulate at sites of chronic inflammation such as atherosclerosis and rheumatoid arthritis and have been shown to possess both pro- and anti-inflammatory properties. For example, while oxPLs, such as oxidised palmitoyl-arachidonyl-phosphatidyl-choline (ox-PAPC), increase induction of monocyte-endothelial interactions and synthesis of interleukin-8 and monocyte chemokine attractant protein-1, they have also been shown to down-regulate inflammatory Toll-like receptor- (TLR) 2 and 4 signalling. As classical (*ie* myeloid differentiation factor-88 dependent) TLR signalling has recently been shown to be a potent regulator of atherogenesis, we investigated whether oxPAPC, mmLDL or oxLDL induces classical activation of TLRs 1, 2, 4 or 6. A HEK-293 cell transfection assay revealed no TLR activation by these agents, and, unlike established TLR ligands, oxPAPC failed to induce expression of TNF-α in monocytic THP-1 cells. The specific TLR4 antagonist *Rhodobacter sphaeroides* lipopolysaccharide did not abrogate oxPAPC signalling in endothelial cells. Finally, fractions of oxPAPC generated by HPLC and synthetic POVPC and PGPC did not activate TLR signalling in transfected HEK-293 cells. We conclude that oxPAPC does not induce classical activation of TLR 1, 2, 4 or 6 signalling.

Effect of transcranial magnetic stimulation during 3-nitropropionic acid-induced loss cell and free radical production in the striatum

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3-Nitropropionic acid (3-NPA) induces an experimental model of Huntington's disease characterized by neuronal loss and oxidative stress. This study evaluates the effects of transcranial magnetic stimulation (TMS) on 3-NPA-induced loss cell and oxidative stress. Male Wistar rats were divided into three groups as follows: (i) Control, (ii) 3-NPA, and (iii) 3-NPA+TMS. 3NPA was administered i.p. at a dose of 20 mg/kg BW for 4 consecutive days in DMSO (0.1%), whereas TMS was applied to 60 Hz and 0.7 mTesla daily for 4 days, beginning after last injection of 3-nitropropionic acid. The oxidative stress by 3-NPA was confirmed by a high level of lipid peroxidation products ($P<0.001$) in striatum synaptosomes and an enhancement in hydroperoxides production in brain ($P<0.001$), as well as by a decrease in total radical-trapping antioxidant potential (TRAP) levels in brain and loss cell in the striatum. These changes were reverted by application of TMS. In summary: (i) 3-NPA induces oxidative stress and neuronal death; and (ii) TMS decreases oxidative stress and loss neuronal induced by 3-NPA. These data indicate the beneficial and neuroprotective effect of TMS.

The authors thank Universidad Nacional Autónoma de México (UNAM) for technical support

Effect of antioxidants and inhibitors of NAD(P)H oxidase and tyrosine kinase activities on glucose uptake in a megakaryocytic cell line

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According to a newer view, deliberate reactive oxygen species (ROS) generation occurs not only in phagocytes but also in other cell types, in which ROS function in cell signalling and metabolism. We have recently studied the relationship between basal level of intracellular ROS and Glut1 activity in a human megakaryocytic cell line, B1647. Glut1 is the transporter isoform responsible for the basal glucose uptake in many cell types and is subjected to "acute" regulation by several metabolic and oxidative stresses. Since the evidence supporting the concept of ROS as signalling molecules is based on the observation that antioxidants and inhibitors of ROS-generating systems block specific physiological responses, we tested the effects of EUK-134, a synthetic SOD and catalase mimetic, on glucose uptake and intracellular ROS level, and we showed that ROS are important to maintain the activation of glucose transport mediated by Glut1. In order to confirm the observed effect of EUK-134 and to obtain information about which reactive species might be involved in glucose uptake, we tested the effect of several ROS scavengers. Results show that different species are involved in this activity. Data obtained in the presence of NAD(P)H inhibitors suggest that a possible ROS generation site could be this membrane-bound enzymatic complex, similar to the phagocytic one. The effects of tyrosine kinase inhibitors and antioxidants show the importance of phosphorylation process in the regulation of Glut1 activity and that a possible target of ROS as molecular signal are protein phosphatases, as reported in literature.

Platelet amyloid precursor protein metabolism in the mild cognitive impairment

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Introduction: The amyloid precursor protein (APP) is proteolytically processed by a beta-secretase (BACE) and a gamma-secretase to produce the pathogenic β-amyloid peptide, main component of senile plaques and a hallmark in Alzheimer's disease (AD). APP is expressed in the brain and in platelets, which contain the same APP processing enzymes found in neurons. Patients with AD show an alterations of APP pattern forms expression in platelet compared with control subjects. The aim is to investigate if the APP processing in platelet could be a biomarker for the diagnosis of preclinical AD and in predicting the progression from mild cognitive impairment (MCI) to AD. *Methods*: The present study was carried out in patients with MCI and patients with AD. Both groups of patients were compared to control group. Western Blot analysis of presenilin and APP were performed on platelet homogenates. Moreover, BACE and gamma-secretase activities were measured by fluorimetric methods in platelets of the same pool of subjects. *Results*: The results showed a significant increase in platelets BACE and gamma-secretase activities in patients with MCI and patients with a diagnosis of AD versus control group. These increased are related with the processing of APP found in these groups of patients. *Conclusions*: According to these observations, platelets can be considered as a source of human biological material available for the study of the mechanisms and the evolution of the biochemical processes related with AD. This observations suggests that APP processing could be a clinical biomarker which might be of helpful value in the diagnosis of AD, and also that MCI is associated with an early stage of AD.

Oxidative damage in acute ischemic stroke patients: A relationship with paraoxonase activity

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Recent studies have demonstrated that stroke is associated with oxidative damage (Polidori MC et al 1998) and a decrease in the levels and activity of antioxidant molecules or cellular enzymes (Sanchez C et al. 2004; Sudha K et al. 2004). To further investigate the relationship between oxidative stress in ischemic stroke, we studied the activity of paraoxonase (PON1) and the levels of lipid hydroperoxides in plasma of acute-ischemic stroke patients from Stroke Unit of Ancona (n = 40), with respect to age-sex-matched controls (n = 50). PON1, a calcium-dependent esterase associated with high density lipoproteins (HDL), plays an important role in the protective effect exerted by HDL against oxidative damage of plasma lipoproteins and cell membranes (Watson AD et al. 1995; Ferretti G et al.2004). Our results demonstrated, for the first time, that the activity of PON1 in plasma of acute-ischemic stroke patients is significantly lower with respect to controls (981.13 ± 127.06 U/mL and 2316.9 ± 225.26 U/mL, respectively, $p < 0.001$). Moreover, the levels of lipid hydroperoxides in plasma from the patients are higher with respect to controls (6.95 ± 0.19 micromol/L and 2.81 ± 0.17 micromol/L, respectively, $p < 0.001$). The negative correlation established between the individual values of PON1 activity and the plasma levels of lipid hydroperoxides confirm the relationship between paraoxonase activity and lipid peroxidation both in healthy subjects ($r = 0.877$, $n = 50$, $p < 0.001$) and ischemic stroke patients ($r = -0.7$, $n = 40$, $p < 0.001$). In conclusion, our study confirms an increase of oxidative stress related with a decrease of antioxidant properties in acute-ischemic stroke patients.

Serum γ-glutamyltransferase: A new factor in atherosclerotic plaque progression?

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Background. A correlation exists between serum gamma-glutamyltransferase (GGT) activity and cardiovascular diseases. As for myocardial infarction, GGT-associated risk disappears after angioplasty, suggesting a connection of serum GGT with plaque instability (Emdin et al. 2001). GGT is able to catalyze LDL-lipoprotein oxidation in vitro in presence of GSH and ferric ions (Paolicchi et al. 1999) and catalytically active GGT is present in cerebral (Emdin et al. 2002) and coronary (Paolicchi et al. 2004) plaques. *Aim and methods*. The hypothesis that GGT found within the plaque may derive from plasma, thus explaining the link between serum GGT and plaque instability, was tested in atheromas obtained by endoarteriectomy. Serum and plaque GGT were characterized by molecular exclusion, anion exchange chromatography and immunoblot. GGT expression was studied by RT-PCR. Free and protein-bound low mol. wt. thiols were analyzed by HPLC. *Results*. i) Part of plaque GGT derives from serum; ii) GGT gene transcription also occurs in plaque tissue; iii) serum GGT is partly associated with LDL; the proportion of LDL-associated GGT is significantly lower in ischemic subjects; iv) cysteinyl-glycine, produced during GGT activity, is present in plaques. *Conclusions*. Serum GGT contributes to GGT activity of the plaque. Redox effects of GGT activity are detectable in the plaque contest. It is thus possible that influx of GGT from serum, likely in association with LDL, may contribute to plaque progression and instability.

Studies supported by MIUR-PRIN and FIRB grants.

Effect of calorie restriction on cellular and molecular modifications of rat arterial wall during ageing

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Many theories have been advanced to account for the ageing process, among which the free radical theory deserves much attention. Many studies have shown a frequent association between tissue fibrosis and oxidative stress and fibrosis may be considered a significant index of tissue ageing. Several studies have shown that calorie restriction (CR) keeps many physiological processes at youthful state until a very advanced age. However, the anti-ageing mechanisms of CR are not yet clear. We have previously shown in the aortae from elderly versus young rats a significant increase in oxidative stress, in terms of fluorescent adducts between lipid peroxidation-derived aldehydes and tissue proteins, and fibrosis, in terms of transforming growth factor β1 (TGFβ1) and collagen levels. In parallel we have shown a progressive age-related increase of c-Jun N-terminal kinase and p38 activity. CR reversed all phenomena. Now we show that CR similarly reverses the age-related morphological alterations of aorta wall cell composition. CR also protects against the age-associated increase of AP-1 DNA binding activity and the AP-1-dependent increase of vimentin gene expression. These data strongly suggest that CR may protect against age-related fibrosis through a decrease of oxidative reactions and a consequent minor transcription of genes involved in the fibrosis process (*e.g.* TGFβ1), mediated by a decrease of AP-1 activation.

The role of iron chelation in the protection offered by flavonoids against hydrogen peroxide-induced cellular DNA damage

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The ability of a number of flavonoids belonging to the flavone, flavonol, flavanone and flavan-3-ol subclasses to protect cellular DNA from H_2O_2-induced single strand breaks and the underlying molecular mechanisms were investigated in this work. Formation of single strand breaks on nuclear DNA, after exposure of Jurkat cells to continuously generated H_2O_2 in the presence or absence of the flavonoid compounds, was evaluated by the comet assay (single cell gel electrophoresis). The results indicate the following structural requirements of flavonoids for effective DNA protection: a) the ortho-dihydroxy structure either in ring A or B; b) the hydroxyl moiety on position 3 in combination with the oxo-group at position 4; and c) the presence of a C2,C3 double bond in ring C. In contrast to free flavonoids, the ability of complexes $[Fe^{2+}]$/[flavonoid] to protect nuclear DNA was decreased as the ratio increased, and the complex was completely inactive when the ratio reached a certain value. Moreover, it was observed that several of the flavonoids tested were able to remove iron from calcein loaded into cells, and that this property was in excellent correlation with their ability to protect DNA ($r^2 = 0.97$). The antioxidant (electron donating) capacities of the same flavonoids were also evaluated by a conventional method, but not correlation with their DNA protective abilities was established even though their membrane penetrating abilities were taken in account. In conclusion, the results presented in this work strongly support the notion that intracellular iron chelation is the most likely mechanism by which flavonoids protect cells against H_2O_2-induced DNA damage.

Implication of hydrogen peroxide in the molecular mechanisms of apoptotic cell death

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The molecular mechanisms of hydrogen peroxide-induced apoptotic cell death have been studied in the present investigation. Jurkat T-cells in culture (1.5×10^6 cells per ml) were exposed either to continuously generated H_2O_2 by the action of the enzyme "glucose oxidase" (100 ng per ml, able to generate 2.0 ± 0.2 μM H_2O_2 per min) or to a bolus addition of 0.25 mM of the same agent. In the first case, it seems likely that steady state conditions were prevailing, while in the latter H_2O_2 was removed by the cellular defense systems following first order kinetics. When a variety of markers for apoptotic cell death (DNA cell content, DNA laddering, activation of caspases, PARP cleavage) were examined 6 hours later, only the bolus treatment was able to induce apoptosis, while the continuous presence of this agent inhibited the appearance of these markers. Apart from the bolus addition, initiation of apoptosis by the Fas receptor pathway was also inhibited by the continuous presence of H_2O_2 during the apoptotic process. Careful examination of the consecutive steps that lead to apoptosis revealed that the process was inhibited at the level of caspase-9 autoactivation. The exact molecular mechanism of this inhibition is under investigation. Moreover, the ultimate fate of cells which were unable to activate the pathway of execution caspases due to the presence of H_2O_2 was also examined and found to follow a caspase-independent pathway in which translocation of apoptosis inducing factor (AIF) from mitochondria to nucleus is involved. These observations indicate that H_2O_2 may modulate the apoptotic process leading to different types of cell death. Whether this modulation of death-type may be connected or influence certain pathological conditions, is still unknown.

Olive oil phenolic fraction and evaluation of its antioxidant capacity

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The health promoting properties of olive oil are due to its unique profile of phenolic fraction along with high amount of squalene and oleic acid. The protective effects of phenolic compounds can be attributed to their strong antioxidant activity, besides their antiproliferative, antihemorragic and anti-inflammatory properties. The concentration of phytochemical compounds in olive oil depends on several factors, including: cultivar, soil, climate, production procedures and storage conditions. In the present study, the total phytochemical content and its antioxidant capacity were measured in 6 olive oil samples from different cultivars, locations and sources. The total polyphenols determination and the detection and quantification of the single polyphenols, revealed that home-made olive oil samples presented various profiles and contents of phenolic fraction, probably due to the differences of variety and location. Olive oil samples from the same variety, extracted with the same technology, and taken from the same region, but harvested and produced in two different years, 2003 and 2004 respectively, showed a different phenolic profile. The 2003 sample was richer of simple phenolic acids, while the 2004 presented a higher amount of complex polyphenols (secoiridoids). At the same time, the latter showed a minor antioxidant activity, measured by the Oxygen Radical Absorbance Capacity Assay (ORAC), than the 2003 sample.

Evaluation of quercetin cytoxicity on human colon tumor cell line and its possible role as chemoresistance modulator

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The development of the resistance to antineoplastic drugs is a major issue in the cancer treatment. Multidrug Resistance (MDR) of cancer cells is often associated with over-expression of P-glycoprotein (P-gp), a plasma membrane transporter. Recently, flavonoids, minor constituents of diet responsible for several biological activities, such as strong antioxidant and antiproliferative properties have been evaluated as MDR modulators with controversial results. Several studies have displayed that polyphenols in certain conditions can act as pro-oxidants, showing cytotoxic and mutagenic effects. We have evaluated the effects of quercetin on tumor cell proliferation and its potential effectiveness as MDR reverser influencing the cytoxicity of antineoplastic drugs (doxorubicin). LoVo wild-type, a human colon adenocarcinoma cell line and LoVo Dx, a multidrug resistant variant cell line, in the presence of quercetin showed a concentration and time-dependent susceptibility to flavonoid toxicity. The cell line WT showed a decrease in viability after 24 hours of quercetin treatment (IC_{50} = 70 μM), while the cell line Dx, were less affected (IC_{50} = 200 μM). With respect to the MDR modulating activity, quercetin was found not to be a P-gp inhibitor compared to the well established P-gp reversers, Verapamil and Cyclosprin A. Conversely, quercetin protected both cell lines against doxorubicin toxicity.

Cytoprotective effect of vitamin E against D-galactosamine-induced cell death is more related to transcriptional than antioxidant regulatory properties in cultured human hepatocytes

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Introduction: D-GalN induces oxidative stress, nitric oxide production and cell death in hepatocytes. Vitamin E regulates oxidative stress in different experimental models. The aim of the study was to determine if vitamin E reduces cell death in cultured human hepatocytes. *Materials and Methods:* Hepatocytes were isolated from human liver resections. Vitamin E (50 μM) was added 10 h after D-GalN (40 mM). Apoptosis and necrosis were assessed by caspase-3 activation and DNA fragmentation, and lactate dehydrogenase (LDH) release. Oxidative stress was evaluated using dichlorofluorescein. NF-*k*B activation was determined by radioactive EMSA. The expression of inducible nitric oxide synthase (iNOS) and CYP3A4 was quantified by real-time RT-PCR. *Results:* Vitamin E reduced all the parameters related to apoptosis and necrosis induced by D-GalN in cultured hepatocytes. Vitamin E was not able to reduce intracellular reactive oxygen species produced by D-GalN. Nevertheless, vitamin E reduced NF-*k*B activation and iNOS expression, and enhanced the expression of CYP3A4. *Conclusions:* 1) D-GalN induced oxidative stress and cell death. 2) Vitamin E reduced cell death induced by D-GalN. 3) Vitamin E did not prevent intracellular oxidative stress. 4) Vitamin E decreased iNOS expression and enhanced CYP3A4 expression in cultured human hepatocytes.

Kaempferol and trans-resveratrol differently affect MAP kinases activation and cell viability of human breast cancer MCF-7 cells

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Polyphenols represent a large class of plant-derived molecules with a general chemical structure that allows acting as potent free radical scavengers. They are long been recognized to possess several therapeutic activities ranging from anti-thrombotic to antioxidant. Moreover, the capability of polyphenols to act as reducing or oxidizing molecules depends on the presence of environmental metals and on the concentrations used. In this work we demonstrated that the flavonol kaempferol (Kp) and the stilbene trans-resveratrol (t-Rv) were able to commit human breast cancer MCF-7 cells to death with the feature of apoptosis. Mainly, we evidenced a pivotal role of the mitochondria in this phenomenon as cytochrome c release into the cytosol was found after the treatment. We further showed that both Kp and t-Rv were able to differently affect cellular redox state. In particular, t-Rv induced an early production of reactive oxygen species (ROS) and oxidation of cellular macromolecules, whereas Kp treatment was principally associated with glutathione redox unbalance through the formation of the intramolecular disulfide GSSG. This different mode of action was mirrored by the different activation of the pro-apoptotic members of the mitogen activated protein (MAP) kinase family, JNK and p38MAPK that are mainly induced during t-Rv and Kp respectively. The results obtained demonstrate a pro-apoptotic and anti-tumor activity for t-Rv and Kp, and suggest different activation of MAP kinases in response to different types of oxidative stress.

This work was supported in part by: MIUR, FIRB and Ministero della Salute Links: filomeni@bio.uniroma2.it and ciriolo@bio.uniroma2.it

Ethanol modulation of nitric oxide production evoked by stimulation of glutamate receptors in hippocampal slices

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Nitric oxide (•NO) is an intercellular neuromodulator, implicated in a number of neuronal events including neuronal plasticity, learning, memory formation and neuronal degeneration or survival. Increased activity of the glutamatergic NMDA receptor (NMDAr) is the dominant, but not the only, mechanism by which •NO is generated in the brain. Glutamatergic AMPA receptors (AMPAr) activation can also result in •NO production, and we have provided evidences for the concentration dynamics of •NO following NMDAr and AMPAr activation in the hippocampus. Attempts to regulate •NO production via glutamate receptors have been investigated and NMDAr have been shown to exhibit special sensitivity to the effect of ethanol, a drug capable of inducing marked behavioral effects. Despite its many intracellular effects, evidences indicate that it can inhibit •NO production in NMDA-stimulated cell cultures or tissue slices. In light of this, we investigated the modulatory effects of sub-toxic levels of ethanol on the dynamics of endogenously-produced •NO in functional hippocampal slices using home-made •NO microsensors. Amperometric recordings after local injection of both NMDA and AMPA indicate that ethanol causes a marked decrease in •NO production after NMDAr activation, and that this inhibition is both time- and concentration-dependent. The results also indicate that this inhibitory effect is lost upon AMPAr activation, suggesting a selective modulatory effect of ethanol on glutamate receptors.

Vitamin E at high doses improves survival, neurological performance and brain mitochondrial function in aging male mice

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Male mice receiving vitamin E (5.0 g α-tocopherol acetate/kg of food) from 28 wk of age showed a 40% increased median lifespan, from 61 ± 4 wk to 85 ± 4 wk, and 17% increased maximal lifespan, whereas female mice equally supplemented exhibited only 14% increased median lifespan. The α-tocopherol content of brain and liver was 2.5-times and 7-times increased in male mice, respectively. Vitamin E-supplemented male mice showed a better performance in the tightrope (neuromuscular function) and the T-maze (exploratory activity) tests with improvements of 9-24% at 52 wk and of 28-45% at 78 wk. The rates of electron transfer in brain mitochondria, determined as state 3 oxygen uptake and as NADH-cytochrome c reductase and cytochrome oxidase activities, were 16-25% and 35-38% diminished at 52-78 wk. These losses of mitochondrial function were ameliorated by vitamin E supplementation by 37-56 % and by 60-66 % at the two considered time points. The activities of mtNOS and Mn-SOD decreased 28-67 % upon aging and these effects were partially (41-68 %) prevented by vitamin E treatment. Liver mitochondrial activities showed similar effects of aging and of vitamin E supplementation, although less marked. Brain mitochondrial enzymatic activities correlated negatively with the mitochondrial content of protein and lipid oxidation products ($r^2 = 0.58$-0.99, $p < 0.01$), and the rates of respiration and of complex I and IV activities correlated positively ($r^2 = 0.74$-0.80, $p < 0.01$) with success in the behavioral tests and with maximal lifespan.

UV-light-induced heme oxygenase-1 expression in skin is mediated by oxidized phospholipids

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Skin cells respond to long-wave UVA (UVA-1) irradiation by expression of stress-response genes such as heme oxygenase-1 (HO-1). However, the mechanism of UVA-mediated HO-1 induction is not clear. UVA and singlet oxygen are known to mediate lipid peroxidation and we and others have previously shown that oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (OxPAPC) induced HO-1 synthesis in vascular and immune cells. Here we show that OxPAPC induces HO-1 expression in skin cells and that oxidation of PAPC with UVA-1 but not UVB leads to formation of lipid oxidation products that induce HO-1 expression. Using mass spectrometry we identify UV-oxidation products that have biological activity as epoxyisoprostane-PC. Our data also suggest that the oxidation of PAPC by UVA-1 is a singlet oxygen-dependent mechanism. Staining of dermal fibroblasts with EO6 antibodies demonstrates UVA-1-dose-dependent formation of oxidation specific epitopes of phosphatidylcholine phoshpolipids. Thus we present a novel mechanism of UVA-1 induced gene regulation that is mediated via phospholipid oxidation products. We show for the first time that lipid mediators that influence gene expression and have immunomodulating effects can be directly generated in the skin upon UVA-1 irradiation. Further analysis will reveal the contribution of these lipid mediators to the response of skin to UVA-1 and their role in UVA-1 phototherapy of inflammatory skin diseases.

Study of antioxidant propertiesof *Berberis vulgaris* fruit extract on the proliferation of human liver cancer cell line (HepG2)

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Normal biochemical processes in human body may produce free radicals. These free radicals can, in turn, lead to oxidative stress related disease. This study examined the antioxidant activity of Berberis vulgaris fruit extract and its cytotoxic effect on human liver cancer cell line (HepG2).The antioxidant activity of Berberis vulgaris fruit extract (BFE) was assay by β-carotene bleaching and 1-diphenyl-2-picrylhydrazyl (DPPH) assay. The screening of cytotoxic effect was carried out by the microculture tetrazolium salt (MTT) assay on the human liver cancer cell line (HepG2). The BFE with concentration of 5-140 µg/ml was used. The control group cell was without any treatment. Intracellular alkaline phosphatase (ALP) activity is determined by p-nitrophenyl phosphate. The concentration of 5 µg/ml was chosen for this test. In β-carotene bleaching, ascorbic acid showed the mean total antioxidant activity of 96.16 ± 5.09%, followed by BHT (66.71 ± 2.52) and BFE (59.91 ± 8.64). In DPPH, the EC50 of ascorbic acid was 0.252 ± 0.000 mg/ml, BHT (0.612 ± 0.009 mg/ml) and BFE (0.685 ± 0.033 mg/ml). The IC50 of BFE was found 106.0 zzzv 10.1 µg/ml. Beside reduction in cell proliferation the crude extract was capable of enhancing the intracellular protein content in cell cancer line by one fourth while intracellular alkaline phosphatase activity increased by 7 fold. The results showed that processed commercial Berberis vulgaris exhibited antioxidant properties, has the ability of reducing cell viability and may had the potential of enhancing the ALP activity probably through structural changes.

Harmful effect of methylparaben on skin keratinocyte

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For many years, methylparaben (MP) has been empirically used as an excellent preservative for foods, cosmetics and medicines in order to keep them from deterioration. However the precise effect of MP on skin keratinocyte in combination with UVB exposure is not known. In the present study, we investigated the harmful effect of methylparaben (MP) on normal human skin keratinocyte. To this end, HaCaT cells were used as a normal skin keratinocyte. MP (0.003, 0.03, 0.3 %) were added to HaCaT cell and cultured for 6 hrs or 24 hrs. In some experiments, MP-treated cells were exposed to UVB (15 mJ/cm^2, 30 mJ/cm^2). In order to quantify the cellular viability, we used MTT assay. The way of cellular death was qualified by using fluorescent microscopy and flowcytometry. Oxidative stress and nitric oxide (NO) production in HaCaT cells were measured by the oxidation of dihydrorhodamine-123 and the nitration of 4-amino-5-methylamino-2',7'-difluorescein diacetate respectively. As a result, low concentration of MP did not affect cellular viability, oxidative stress and NO production in HaCaT cells. Low dose of UVB also has no or little effect on these parameters in HaCaT cells. However the same amount of UVB exposure significantly increased cell death, oxidative stress and NO production in MP-contditioned HaCaT cells. These results indicate that MP, which has been thought to be a safety preservative in cosmetics, might have harmful effect on human skin when used under the sunlight.

Molecular mechanisms of action of genistein and dadzein on glutathione induction in endothelial cells

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It has been hypothesized that the beneficial effects of soy consumption may be linked to the antioxidant capacity of the isoflavones themselves and/or their ability to maintain the redox equilibrium of endothelial cells through stimulating increases in cellular GSH levels. We have investigated the effect of genistein and daidzein on oxidative injury, glutathione levels and γ-GCS expression in an endothelial cell line. EA.hy 926 cells pre-treated with genistein (25 or 50μM: 24h) showed resistance to hydrogen peroxide (50 μM; 6h) induced cytotoxicity. Daidzein appeared to exacerbate the effects of peroxide. A significant increase in glutathione levels was observed after treating the cells with daidzein at 25 and 50μM for 48h. However, genistein, at the same concentrations, showed a significant depletion of glutathione. In order to elucidate the possible mechanisms behind these effects both the gene and the protein expression of γ-GCS-HS (the γ-GCS catalytic subunit) were investigated. Genistein and daidzein (25 or 50 μM: 24 or 48h) did not induce γ-GCS-HS protein expression. After 6h and 12h treatments at the same concentrations, gene expression was also unaffected. These results indicate that the two isoflavones have opposite effects with respect to counteracting hydrogen peroxide cytotoxicity and glutathione levels and suggest that they do not seem to exert these effects by modulating γ-GCS-HS expression.

Reaction of nitrogen monoxide with glutathione thiyl radical – A kinetic study

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The biosynthesis of *S*-nitrosoglutathione (GSNO) is proposed to involve the reaction of nitrogen monoxide (NO•) with the glutathione thiyl radical (GS•) (1). Therefore the reaction GS• + NO• → GSNO (reaction 1) was studied under various conditions.

The laser flash photolysis at 266 nm of GSNO in NO•-saturated water lead to GS• + NO•. Their recombination (with a rate constant $k_1 \leq 6.3 \times 10^7$ $s^{-1}M^{-1}$) was not quantitative. With pulse radiolytic reduction of GSSG by H•/e-aq in 95% NO• saturated water a rate constant of $k_1 \approx 3 \times 10^7$ $M^{-1}s^{-1}$ was determined. These results and the low bioavailable concentrations of the participating species ([NO•] ≤ 10^{-7} M) indicate that the radical formation pathway is not probable: The decay of GS• by intramolecular H• transfer (2) is faster than 10^3 s^{-1}. Under such conditions, the yield of GS• + NO• is less than 1%.

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The dietary soy flavonoid genistein abrogates tissue factor induction in endothelial cells by the atherogenic oxidized phospholipid oxPAPC

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Tissue Factor (TF) plays a pivotal role in the generation of thrombin within atherothrombotic disease. The oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC), an active compound of minimally oxidized low-density lipoprotein (MM-LDL), has been demonstrated to induce TF in endothelial cells (EC). The dietary soybean isoflavonoid Genistein has been claimed to reverse several processes leading to atherosclerosis and related cardiovascular events via binding to the estrogen receptors, generating nitric oxide (NO) or inhibiting tyrosine kinase dependent pathways. Genistein abrogated oxPAPC-induced TF activity in HUVEC to basal levels, as measured by clotting assay, and further downregulated oxPAPC-induced antigen expression in the flow cytometry assay and mRNA levels in the real time PCR assay. As shown by Western blotting of tyrosine phosphorylated proteins and experiments with the estrogen receptor inhibitor ICI 182.780 and NO-synthase inhibitor N(omega)-nitro-l-arginine methyl ester (L-NAME), the effect is primarily mediated via inhibition of phosphorylation of intracellular proteins and not by binding to the estrogen receptor or generation of NO as active metabolite. Genistein reduces oxPAPC-induced TF expression and thereby the prothrombotic phenotype of EC, further substantiating and explaining the beneficial effects of dietary genistein in preventing atherosclerosis and related cardiovascular events.

Glutaredoxin 2, oxidative stress and cell death

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Glutaredoxins (Grxs) are versatile thiol disulfide oxidoreductases. Unlike other Grxs, human mitochondrial Grx2 can receive electrons from GSH and thioredoxin reductase enabling catalysis during oxidative stress [1]. HeLa cells with silenced expression of Grx2 are dramatically sensitized to cell death induced by the oxidative stress-inducing agents doxorubicin and phenylarsine oxide [2]. On the other hand, over-expression of Grx2 in HeLa cells decreased the susceptibility to apoptosis by preventing loss of cardiolipin and inhibition of cytochrome c release [3]. In-depth spectroscopic analysis revealed the presence of a four cysteine-coordinated, non-oxidizable [2Fe-2S] cluster in fresh Grx2 preparations [4]. Two cysteinyl groups outside the active site were suggested to complex the cluster and dimerize Grx2. Co-immunoprecipitation of 55Fe with Grx2 from human cell lines indicated the presence of the cluster in vivo. While holo-Grx2 is enzymatically inactive, redox-induced desintegration of the cluster generates active apo-Grx2, which contains a second, structural disulfide instead of the cluster. The properties of Grx2 are highly adopted to variable redox conditions enabling the maintenance of mitochondrial redox homoeostasis upon oxidative stress.

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Nitrolinoleic acid (LNO_2) induces HO-1 via transcriptional and translational mechanisms in pulmonary epithelial cells

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It is well established that lipid nitration increases under conditions of stress. Here we show that the lipid nitration product nitrolinoleic acid (LNO_2) is increased in the pulmonary edema fluid of patients with ARDS. We hypothesize that LNO_2, although a by-product of inflammation, is involved in pulmonary-protection by inducing the cytoprotective enzyme heme oxygenase-1 (HO-1). We tested this hypothesis both *in vitro* using human (A549) and rat (L2) cells treated with LNO_2 (5 to 50 μ M), and *in vivo* using rats injected with LNO_2. HO-1 induction by LNO_2 was confirmed in the cell lines and in the rat lung epithelial cells by immunohistochemistry. Western blotting revealed that the HO-1 protein increased significantly 4 h post-incubation and was preceded by an increase in HO-1 mRNA suggesting transcriptional control of HO-1 by LNO_2. HO-1 activity, a functional measure of HO-1, was significantly increased by LNO2 in both cell lines. Phosphoryation of ERK, JNK and p38 MAPK preceded the induction of HO-1 suggesting MAPK regulation of HO-1 mRNA and protein. Interestingly, LNO_2 also activated eIF2-α, a key, redox-sensitive regulator of translation. This suggests a novel mechanism by which LNO_2 increases HO-1.

Iron prevents ascorbic acid (Vitamin C) induced hydrogen peroxide accumulation in copper-contaminated drinking water

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Ascorbic acid (vitamin C) induced hydrogen peroxide (H_2O_2) formation was measured in household drinking water and metal supplemented Milli-Q water by using the FOX assay. Here we show that ascorbic acid readily induces H_2O_2 formation in Cu(II) supplemented Milli-Q water and poorly buffered household drinking water. In contrast to Cu(II), iron was not capable to support ascorbic acid induced H_2O_2 formation during acidic conditions (pH: 3.5-5). In 12 out of the 48 drinking water samples incubated with 2 mM ascorbic acid, the H_2O_2 concentration exceeded 400 μM. However, when trace amounts of Fe(III) (0.2 mg/l) was present during the incubation, the ascorbic acid/Cu(II)-induced H_2O_2 accumulation was totally blocked. Of the other common divalent or trivalent metal ions tested, that normally are present in drinking water (calcium, magnesium, zinc, cobalt, manganese or aluminum), only calcium and magnesium displayed a modest inhibitory activity on the ascorbic acid/ Cu(II)-induced H_2O_2 formation. Oxalic acid, one of the degradation products from ascorbic acid, was confirmed to actively participate in the iron induced degradation of H_2O_2. Ascorbic acid/Cu(II)- induced H_2O_2 formation during acidic conditions, as demonstrated here in poorly buffered drinking water, could be of importance in host defense against bacterial infections. In addition, our findings might explain the mechanism for the protective effect of iron against vitamin C induced cell toxicity.

Microsomal activity measured by ^{13}C-methacetin breath test is associated with oxidative stress in nonalcoholic fatty liver disease (NAFLD)

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Nonalcoholic fatty liver disease is one of the most prevalent hepatic diseases around the world; its progression has been related to an increased microsomal function and oxidative stress. The ^{13}C-methacetin-breath test (MBT) allows a functional quantitative assessment of the hepatic mass. The aim of this work was to evaluate the relationship between MBT values and oxidative stress serum markers in patients with NAFLD. 67 patients with NAFLD and 20 healthy controls (HC) were studied. NAFLD patients were classified as having only steatosis (n = 26) or nonalcoholic liver disease (NASH, n = 41) according to Matteoni et al.1999. MBT was performed with 75mg of ^{13}C-methacetine, and the $^{13}CO_2$ in the expired air was measured by nondispersive infrared spectrophotometry. The cumulative recovery of $^{13}CO_2$ was calculated at 120 minutes (MBT120min). Erythrocyte glutathione peroxidase activity and lipoperoxidation (LPO) were assessed by spectrophotometric methods; plasma antioxidant vitamins were measured by HPLC. MBT120min was reduced in the NAFLD group (38.3 ± 8.7% for HC and 28.8 6.5% for NAFLD, p = 0.001). MBT120min values correlate with LPO (r = -0.308, p = 0.018) and glutathione peroxidase activity (r = 0.257, p = 0.048). Within this NAFLD population, no differences in MBT and serum oxidative stress markers were found among patients with NASH and those with steatosis only.

FAPESP (02/05260-6).

The antioxidant bilirubin improves the clinical outcome in a mouse model of endotoxemia

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The inducible isoform of heme oxygenase HO-1, plays important roles in attenuating tissue injury and in the resolution of acute inflammation. HO-1 catabolises the degradation of heme into carbon monoxide, free iron, and biliverdin. The latter is quickly converted to bilirubin that has been shown to potently quench production of reactive oxygen species. In this study we investigated the possible cytoprotective role of bilirubin, employing a mouse model of endotoxemia. Mice were challenged with lipopolysaccharide (LPS) and the effects of intravenously administered bilirubin were assessed. A clinical score, assessing eye inflammation, stool consistence, fur quality and activity upon moderate stimulation was applied. Bilirubin significantly improved the clinical score and LPS-induced anorexia, and attenuated weight loss. Furthermore animals that received bilirubin recovered from LPS-shock within 24 hours. Immunohistological analysis of different organs revealed a reduced leukocyte-endothelial interaction and a reduced expression of ICAM-1 in endothelium upon bilirubin treatment. These data show that bilirubin attenuates tissue injury induced by endotoxin and suggest that anti-inflammatory and cytoprotective properties of HO-1 are mediated through the action of bilirubin.

Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 3-nitrotyrosine in urine by HPLC

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Two potential biomarkers that reflect the enhanced generation of reactive nitrogen and oxygen species are 3-nitrotyrosine and 8-hydroxy-2'-deoxyguanosine (8-OH-2'dG). 3-nitrotyrosine is formed in the organisms by nitration of tyrosine residues via peroxynitrate and is a biomarker of reactive nitrogen species. 8-OH-2'dG is a principal stable marker of hydroxyl radical damage to DNA. We developed a high-performance liquid chromatographic (HPLC) method for the simultaneous determination of 8-OH-2'dG and 3-nitrotyrosine in urine. Analytes are extracted from human urine using solid-phase extraction. The samples are acidified (pH 5-6) using acetic acid. Then, 2 ml of the urine samples are loaded on a C18 SPE catridge previously conditioned with 2 ml methanol and 2 ml water. After washing with 2 ml water and 2 ml of 25 mM ammonium acetate buffer pH 3,5, the sample is eluted with 2 ml of 10% acetonitrile in buffer. The recovery was 83% for 88-OH-2'dG and 70% for 3-nitrotyrosine. A volume of 100 ìl of the extracts is injected into the HPLC system. Isocratic chromatography is performed on a reverse phase column (Betabasic-C18, 5 ìm, 4.6 x 250 mm). The mobile phase consists of 7,5 % acetonitrile and 92,5% Buffer (25 mM ammonium acetate, pH 5,0) at flow rate 1ml/min. The eluents are monitored by UV detection at 278 nm for 3-nitrotyrosine and at 254 nm for 8-OH-2'dG with wavelenth interchange during the run. Retention times are 5 and 7,63 min for 8-OH-2'dG and 3-nitrotyrosine respectively. This method offers an aproach for estimating oxidative DNA and protein damage.

Copper- and magnesium protoporphyrin complexes inhibit oxidative modification of LDL induced by hemin, transition metal ions, and tyrosyl radicals

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The oxidative modification of LDL may play a role in atherogenesis. Thus the identification of antioxidative compounds may be of therapeutic and prophylactic importance. Copper-chlorophyllin (Cu-CHL), a Cu^{2+} -protoporphyrin IX complex, has been reported to inhibit lipid oxidation in biological membranes and liposomes. Hemin (Fe^{3+} -protoporphyrin IX) has been shown to bind to LDL thereby inducing lipid peroxidation. As Cu-CHL has a similar structure as hemin one may assume that Cu-CHL may compete with the hemin action on LDL. Therefore, in the present study Cu-CHL and the related compound magnesium chlorophyllin (Mg-CHL) were examined in their ability to inhibit LDL oxidation initiated by hemin and other LDL oxidizing systems. LDL oxidation by hemin in presence of H2O2 was strongly inhibited by both CHLs. Both chlorophyllins were also capable to effectively inhibit LDL oxidation initiated by transition metal ions (Cu^{2+}), endothelial cells (HUVEC) and tyrosyl radicals generated by myeloperoxidase in presence of H_2O_2 and tyrosine. Cu- and Mg-CHL showed radical scavenging ability as demonstrated by the DPPH- radical assay and estimation of phenoxyl radical generated diphenyl (dityrosine) formation. As assessed by ultracentrifugation the chlorophyllins were found to bind to LDL (and HDL) in serum. The present study shows that copper chlorophyllin and its magnesium analogue could act as potent antagonists of atherogenic LDL modification induced by various oxidative stimuli. As inhibitory effects of the CHLs were found at concentrations as low as 1 μmol/L, which can be achieved in humans, the results may be physiologically / therapeutically relevant.

Oxidative and nitrative DNA damage in inflammation- associated carcinogenesis

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Chronic infection and inflammation are risk factors of a wide variety of cancers. Infection with *Helicobacter pylori* leads to gastric cancer through chronic gastritis. Hepatitis C virus infection causes liver cancer via hepatitis. Inflammatory bowel diseases (IBD), especially ulcerative colitis, are associated with colon carcinogenesis. NO is produced by iNOS expression via NF- κB activation. NO and related reactive species cause DNA damage, which may contribute to inflammation-associated carcinogenesis. We examined the formation of mutagenic 8-nitroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in biopsy specimens obtained from patients of *H. pylori*-induced gastritis and hepatitis C. A double immunofluorescence labeling study demonstrated that 8-nitroguanine and 8-oxodG were strongly formed in the nucleus of gastric gland epithelial cells in gastritis patients with H. pylori infection (Ma et al. BBRC 319: 506, 2004) and hepatocytes in patients with chronic hepatitis C (Horiike et al. J. Hepatol. in press). We also found that 8-nitroguanine and 8-oxodG were colocalized in colonic epithelial cells of IBD model mice and patients with ulcerative colitis (Ding et al. Cancer Sci. 96: 157, 2005). These results suggest that iNOS expression results in DNA damage at the sites of carcinogenesis, regardless of etiology. In conclusion, 8- nitroguanine could be a useful biomarker to evaluate the risk of inflammation-mediated carcinogenesis and the efficacy of the treatment of inflammatory diseases.

Broad-band ultraviolet B phototherapy alters serum thiobarbituric acid reactive substance and nitrite-nitrate levels in psoriatic patients

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Broad-band ultraviolet B (BB-UVB) phototherapy is a widely used therapeutic tool in hyperproliferative skin diseases. Although UVB therapy is known to inhibit nuclear DNA synthesis in the proliferating cells, its systemic effect has not been investigated. We aimed to investigate the lipid peroxidation status (as represented by thiobarbituric acid reactive substance, TBARS) and nitrite-nitrate levels in psoriatic patients (n = 32) who underwent BB-UVB phototherapy and 20 healthy controls. Clinical severity of psoriasis was assessed by the Psoriasis Area and Severity Index (PASI). Blood samples were obtained at the beginning, after 6-10 exposures (1st control) and at the end of the therapy (2nd control) and serum TBARS and nitrite-nitrate levels were evaluated. There was no statistically significant difference in TBARS and nitrite-nitrate levels between psoriatic patients (basal) and healthy volunteers. There was no statistically significant correlation between the disease duration or the disease severity or the total cumulative dose of UVB and serum levels of TBARS and nitrite-nitrate in psoriatic patients. Total nitrite levels in first and second control were significantly higher than basal levels ($p = 0.033$ and $p = 0.005$). TBARS levels in first and second control were significantly higher than basal levels ($p = 0.000$ and $p = 0.026$). There was a negative correlation ($r = -0.576$, $p = 0.039$) between the total nitrite and TBARS levels in psoriatic patients in the second control. Our study shows that chronic UV irradiation may lead to a systemic effect on lipid peroxidation and NO levels and this systemic effect should be taken into consideration in psoriatic patients who have high risk for oxidative stress related diseases.

Metabolism of *trans*-4-hydroxy-2-nonenal (HNE) by murine astrocytes

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Concentrations of the cytotoxic lipid-aldehyde, trans-4-hydroxy-2-nonenal (HNE) are elevated in the brain in Alzheimer's disease. Astrocytes play a crucial role in regulating and supporting neuronal processes; however, their capacity to detoxify HNE is unknown. In this work, we studied the potential phase I (oxidation or reduction) and phase II detoxification (glutathione conjugation) pathways of HNE in murine astrocytes. Astrocytes were exposed to increasing concentrations of HNE (1, 5, and 15 μM). We measured HNE and its phase I metabolites trans-4-hydroxy-2-nonenoic acid (HNA), trans-1,4-dihydroxy-2-nonene (DHN), and phase II metabolites (4-hydroxynonanal-3-yl)glutathione (GSHNE) and (1,4-dihydroxynonane-3-yl)glutathione (GSDHN). Loss of HNE occurred in a time and concentration-dependent manner. At all concentrations of HNE, approximately 80% of the HNE was consumed by 10 minutes. However, the percentage of HNE metabolized to HNA, GSHNE, and GSDHN decreased with increasing HNE concentration such that for 1 μM HNE, 69% of loss could be accounted whereas with 15 μM HNE, only 14% of loss could be accounted for. 10-15% of HNE loss was a result of protein binding. HNA formation was the dominant route of HNE metabolism at 1 μM and 5 μM HNE. The glutathione adducts of HNE were only a small percentage of HNE loss. DHN was not formed. The results demonstrate that HNE is quickly metabolized by astrocytes by multiple, and as yet, unidentified processes.

Different effects of the doxyl nitroxides against the damage to plasma membrane induced by anthracyclines

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Anthracyclines are important anticancer drugs widely applied in chemotherapy of broad spectrum of cancers. Their use is, however, restricted by the acute and chronic cardiomyopathy developed by the appreciable number of patients. Generation of free radicals in redox cycle of anthracyclines is believed to be a main reason for the toxicity of these drugs in relatively unprotected heart myocytes. Different compounds with strong antioxidative properties have been tested for their possible protective effect against cell damage induced by anthracyclines. In this work we investigated the influence of two nitroxides: 5-doxyl stearic acid (5-DS) and 12-doxyl stearic acid (12-DS) on the plasma membrane of cells treated with anthracyclines doxorubicin (DOX) and aclarubicin (ACL). Doxyl acids locate at different depth of the cell membrane and thus can modify membrane properties in different way. We have found that 5-DS by itself modified plasma membrane fluidity in a dose-dependent manner and caused, similar to the investigated anticancer drugs, membrane rigidification, while 12-DS did not influence this membrane property. 5-DS acted synergistically with DOX and ACL and potentated the decrease in membrane fluidity induced by these drugs. 12-DS displayed different effect: in cells treated with ACL decreased membrane fluidity, while in cells treated with DOX opposite effect, namely fluidization of plasma membrane was observed, especially at higher concentrations of the drug and the nitroxide.

Ethanol preconditioning attenuates ischemia/reperfusion-induced leukocyte adhesion in murine small intestine

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Aim. This study aimed to determine whether oxidants act as initiators of late ethanol preconditioning (EPC). *Methods*. On Day 1, ethanol was administered as a bolus to C57BL/6 mice at a dose that produced a peak plasma concentration of 45 mg/dl 30 min after administration and returned to control levels 60 min after ingestion. 24 hrs later, the superior mesenteric artery was occluded for 45 min followed by 60 min of reperfusion. The numbers of fluorescently-labeled rolling and adherent leukocytes were counted in postcapillary venules of the small intestine in sham animals, in mice subjected to I/R alone or EPC+I/R, and in animals treated with Mn-TBAP, oxypurinol, the NAD(P)H oxidase inhibitors PR39 or apocynin, or oxypurinol plus PR39 during the period of EPC on Day 1 followed by I/R on Day 2. In separate groups of mice, oxypurinol or apocynin were also administered 1 hr after ethanol gavage on Day 1, with induction of I/R 24 hours later. *Results*. I/R induced marked increases in leukocyte rolling and adherence, effects that were completely prevented by EPC. Coincident treatment with Mn-TBAP, oxypurinol, PR39, apocynin, or oxypurinol plus PR39 with ethanol attenuated these protective effects of EPC. However, administration of oxypurinol or apocynin 1 hr after ethanol gavage failed to prevent these anti-inflammatory actions of EPC. *Conclusions*. These results indicate that reactive oxygen species formed during the period of ethanol exposure on Day 1 trigger the development of an anti-inflammatory phenotype that renders the small bowel resistant to the proadhesive effects of I/R 24 hours later.

Zinc inhibits enhanced transport of vitamin C during respiratory burst

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During respiratory burst, the NADPH oxidase complex of phagocytes produces superoxide and reactive oxygen species to kill bacteria and other invaders. Differentiated HL-60 cells accumulate large quantities (in the mM range) of vitamin C (ascorbate) when stimulated by phorbol esters (PMA). This increase in vitamin C transport is due to activation of the NADPH oxidase and oxidation of ascorbate to dehydroascorbic acid (DHA). DHA is preferentially taken up by HL-60 cells, reduced back to ascorbate and stored intracellularly to protect the cells from untoward side-effects of the oxidative burst. Upon stimulation with PMA, 1 to 2 nmol/min of superoxide are released by 106 differentiated HL-60 cells as measured by the superoxide dismutase-inhibitable reduction of ferricytochrome c. Zinc and other divalent cations are known to inhibit NADPH oxidase. However, the mechanisms are still not clarified in detail. We found that zinc reversibly inhibits both PMA-stimulated ascorbate uptake and superoxide generation with a half-maximal effect at 20 μM of zinc. When the fluorescent dye 3,3'-dipropylthiadicarbocyanine iodide (diSC3(5)) was used to monitor shifts in membrane potential, we found that depolarization with PMA was prolonged after preincubuation of the cells with zinc. Furthermore, higher residual extracellular ascorbate concentrations were monitored with increasing zinc concentrations indicating that less ascorbate was oxidized and taken up by the cells. Zinc is known to be essential for highly proliferating cells in the human body, especially the immune system. Conversely, we found that high dosages of zinc suppress the respiratory burst and therefore might elicit negative effects on immune cells.

Peroxides infused with TPN solutions lead to accumulation of plasma triglycerides

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Background: Exposure of pareternal nutrition (TPN) to ambient light generates peroxides by reactions involving multivitamins (MVP). Shielding TPN from light limits H_2O_2 levels, vitamin loss, lipid peroxidation, and hepatic steatosis. *Hypothesis*: Peroxides in TPN interfere with lipid metabolism. *Objective*: To evaluate the effects of H_2O_2 and/or photo-protection of TPN on plasma triglycerides (TG) and hepatic lipid metabolism. *Methods:* TG were compared in the 1st 9 d in infants randomized to TPN, light exposed (LE) or light protected (LP). In further infants on fat free TPN, de novo lipogenesis measured by respiratory quotient (RQ > 1) was compared between LE and LP. Effects of H_2O_2 on activities of enzymes involved in lipid synthesis were compared in guinea pigs receiving lipid-free intravenous solutions: control (C) = dextrose; C + H_2O_2; C + MVP (LE); C + MVP (LP). Plasma TG and activities of hepatic acetylCoA carboxylase (ACC) and AMP-activated protein kinase (AMPK) were measued after 4 d. *Results:* Despite similar lipid intakes, TG was 60% higher($p < 0.01$)by days 8-9 with LE. RQ rose ($p < 0.05$) above 1 only in LE. In animals, plasma TG was higher($p < 0.01$)in groups receiving solutions containing peroxides (C + H_2O_2; C + MVP (LE) compared to (C; C + MVP (LP). ACC, was activated by H_2O_2 and inhibited by MVP via its effect on AMPK. *Conclusions:* Higher plasma TG levels in infants receiving light-exposed TPN are explained by a peroxide-induced modification in endogenous lipid metabolism stimulating de novo lipogenesis. Failure to shield TPN solutions from light causes perturbations in lipid metabolism.

Endogenously-produced nitric oxide in hippocampus modulates tissue oxygen profiles

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In the hippocampus, activation of the NMDA receptor may lead to •NO production. Little is known on the dynamics of •NO concentration in tissues, in particular the pathways of its decay. The reaction of •NO with O_2 is slow under the normoxic conditions but may acquire significance for the high oxygen tensions used in the perfusion experimental system, thus misleading •NO dynamics by enhancing its decay rate. •NO competes with O_2 for cytochrome c oxidase, thus regulating mitochondrial respiration. Preliminary experiments address the relation between •NO dynamics and O_2 tensions (PO_2) in hippocampal brain slices. Using carbon fiber microelectrodes we studied the PO_2 profiles in hippocampal slices (400 ìm thick) superfused with artificial cerebral-spinal fluid saturated with 95%O_2/5%CO_2. In the CA1 subregion of the hippocampal slice the core of the tissue (200 to 300 ìm deep) showed an average oxygen tension of 10-20 torr, close to values described in the literature as physiological. We also found that the different subregions are distinct in what concerns O_2 consumption, with CA1 showing the steepest gradient along the tissue depth. Using modified carbon fiber microelectrodes we previously demonstrated that the different subregions are distinct in terms of NMDA evoked •NO transients, with CA1 showing highest levels of •NO production and fastest kinetics of production and removal. Here, by simultaneously recording O_2 and •NO we show that O_2 consumption is blocked when a threshold of •NO is reached. This observation acquires high physiological relevance when considering that •NO regulates tissue O_2 consumption by reversibly inhibiting cytochrome c oxidase.

Funded by FCT: POCTI/BCI/42365/2001

Cardioprotective effects of quercetin and cyanidin-3-*O*-β-glucopyranoside

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Prevention of cardiac cell death associated with myocardial infarction and heart failure is a major area of clinical interest. Polyphenols, thought to be simple radical scavengers, are now receiving significant attention as cytoprotective agents for the prevention of heart diseases due to their ability to modulate oxidative stress-induced signalling pathways. Pathophysiology of ischemic heart disease is multifactorial and a major contributing factor is the loss of myocardial cells which can occur via necrosis or apoptosis. Recent studies have reported that reactive oxygen species (ROS) might regulate apoptosis activating multiple signalling pathways in the heart, including mitogen activated protein kinase (MAPK) pathways, of which c-Jun NH2-terminal protein kinase (JNK) and p38 kinase (p38) are proapoptotic, whereas ERK1/2 is antiapoptotic. Using cultured rat cardiomyocytes we demonstrated that Quercetin (Q) and Cyanidin 3-*O*-β-glucopyranoside (C3G), two antioxidant polyphenols present in the Italian diet, not only protect cardiac cells against oxidative damage, decreasing ROS production, lactate dehydrogenase release and conjugated diene-containing lipids levels, but also counteract apoptotic cell death by decreasing the expression of the active form of the proapoptotic kinases, resulting in a lower caspase-3 activity. Therefore, nutritional assumption of Q and C3G could be useful in the prevention of heart diseases because these polyphenols are potent bioactive molecules, acting both as antioxidants and modulators of cell signaling.

Supported by COFIN 2003 and Fondazione del Monte di BO e RA

Betanin inhibits myeloperoxidase/nitrite-mediated peroxidation of human low-density lipoprotein

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Betanin, the betalain pigment in the plants of the Cariophillalae order, including cactus pear, has recently been reported to be a reducing molecule and a lipoperoxyl radical-scavenger in vitro (1). In addition, it is bioavailable, accumulates in human LDL after ingestion of cactus pear fruits, and protects LDL against copper-induced oxidation in vitro (2,3). Myeloperoxidase (MPO) has been implicated in the in vivo LDL modification and atherogenesis (4). In the presence of nitrite, MPO generates two oxidizing agents, the tyrosyl- and the nitrosyl-radical, that promote LDL oxidation (4). We investigated weather betanin counteracted MPO/nitrite-induced LDL oxidation. Our results indicate that betanin inhibits the MPO/nitrite-induced LDL oxidation in a dose-dependent manner, in the range 1 to 10 microM. When compared to ascorbic acid, a nitrosyl radical-scavenger (4), betanin, was more effective at inhibiting LDL oxidation. The IC50 calculated for betanin (1.4 μM) was more than 10-fold lower than that for ascorbic acid (15.6 μM). Our study shows that betanin protects LDL in an experimental set-up of physiological relevance. In addition, the molecule acts at micromolar concentrations, and appears much more effective than ascorbic acid. Our data collectively indicate a favourable modulation of the oxidation process of LDL and may contribute to explain the beneficial effect of cactus pear fruits (2).

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Gender- and age-related differences in mitochondrial toxicity of β-amyloid peptide

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Introduction: Alzheimer's disease is an age-related disease of multifactorial origin. It is generally accepted that β-amyloid peptide causes neuronal lesions that are at least in part responsible for cellular damage in this disease. There is, however, growing evidence of intracellular toxicity of ß-amyloid (βA) peptide. Its intracellular accumulation is facilitated by ABAD (beta-amyloid biding alcohol deshidrogenase). *Aim*: The aim of this work was to examine the possibility that βA directly increases oxidant production by mitochondria, thus activating the mitochondrial pathway of apoptosis (mediated by the release cytochrome c). Since the incidence of Alzheimer's disease is higher in women than in men the effect of gender of βA peptide induced an increase in oxidant production by mitochondria has also been examined. *Results*: We have shown that βA peptide caused an increase in the rate of oxidant production by isolated mitochondria, but only in young male mitochondria and in old female ones. Initially, these mitochondria were morphologically intact but after one hour of incubation with βA they aggregated. Reduced glutathione (GSH) prevented this aggregation. After 6 hours of incubation, ßA peptide induced a released of cytochrome c from young male and old female mitochondria, but not in young female. *Discussion*: Our results show that the production of free radicals by mitochondria is an early phenomenon in the toxicity of βA peptide. Likewise, we found that it also caused mitochondrial aggregation and the released of cytochrome c, both of which are apoptogenic signals. Young females showed a clear protection from βA toxicity that could be explain by its lesser basal level of oxidative stress.

Dietary polyphenols from red and white wines reduce the oxidative stress associated with inflammation in rats

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In vitro experiments have demonstrated that polyphenols exhibit antioxidant activity. This study was designed to test whether dealcoholized red (DRW)and white (DWW) wines can reduce the oxidative stress associated with inflammation. Rats were fed for 15 d either a control diet or one supplemented with DRW or DWW. Finally, a granuloma was induced by subcutaneous administration of carrageenan. Although DRW showed higher antioxidant activity than DWW, both wines reduced the number of cells recruited into the granuloma pouch. Total phenol content increased while certain oxidative stress parameters decreased in plasma and inflammatory exudate from rats fed with DRW-and DWW-rich diets. Moreover, the concentration of nitrites increased in exudate, possibly related to the increase in the citrulline/arginine ratio. Polymorphonuclear cells from the inflammatory exudate of rats fed dealcoholized wines showed decreased superoxide anion production and increased nitric oxide (NO) production ex vivo. This change in NO resulted from increased expression and activity of inducible NO synthase. Moreover, increased prostaglandin E2 in exudate with DRW was observed, which was possibly related to upregulation of cyclooxygenase-2 protein expression by NO. Our results suggest that the non-alcoholic components of wines not only improve antioxidant status in an inflammatory situation, but also limit cell infiltration possibly through the increase in NO production. These mechanisms could explain the preventive action of polyphenols in the inflammation.

Zinc deficiency impairs, in part, the translocation of activated NF-κB to the nuclei by increasing oxidant levels

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The role of oxidants on NF-κB activation and nuclear translocation associated with zinc (Zn) deficiency was investigated in IMR-32 cells. Cells were incubated in control media or chelated media containing 1.5, 5 or 15 μM Zn, without or with 0.5 mM α-lipoic acid (LA) or 1 mM N-acetyl-L-cysteine (NAC) for 24h. H_2O_2 and total oxidant cell concentrations were higher and total glutathione concentrations were lower in the low Zn groups (1.5 and 5 Zn) compared to control and 15 Zn groups. LA or NAC prevented the increase in H_2O_2 and total oxidants levels, and restored glutathione concentration in the low Zn cells. Zn deficiency induced IkappaB phosphorylation and a high NF-κB-DNA binding activity in total cell extracts. However, the active dimer accumulated in the cytosol, as shown by a low ratio of nuclear/cytosolic NF-κB binding activity. In the Zn deficient cells, a low rate of in vitro tubulin polymerization and of polymerized tubulin content was observed compared to the other groups. The simultaneous incubation of Zn deficient cells with LA or NAC partially restored the nuclear translocation of the active NF-κB. In summary, a decrease in cellular Zn affects NF-κB modulation partially through an increase in cellular oxidants. Oxidants affect both, the early steps in the activation and NF-κB nuclear translocation.

Supported by CONICET (PIP 02120), UC Davis and NIH (HD 01743)

Phosphorylation processes and glucose transport regulation in a human leukaemic cell line

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We have recently shown that reactive oxygen species (ROS) are generated in a megakaryocytic cell line (M07e) in response to cytokines; the ROS increase modulates glucose transport as intracellular messenger in signal transduction. We have also demonstrated that tyrosine phosphorylation and PLCγ play a role in the GLUT1 trafficking, unlike PI3K and PKC.Since the intracellular mechanisms of GLUT1 regulation are still unclear, in this study we tested the effect of some tyrosine kinase (TK) inhibitors to identify if enzymes, such as c-Kit, Src, Syk, AMPK, PKB, MAPK p38 and ERK _, are involved in the glucose transport signalling activated by cytokines and H_2O_2 in a human acute leukaemia (M07e) cell line. To confirm the role of these steps and clarify the enzyme order in the cascade transduction, we investigated the enzyme phosphorylation variations also in the presence of some inhibitors. Incubation with cytokines and H_2O_2 produced a similar increase in the phosphorylation pattern in M07e cells, even if the H_2O_2 effect is less specific. At first we found that a direct change in the phosphorylation on GLUT1 doesn't occur. On the other hand, c-Kit, the stem cell factor (SCF) receptor, was activated either by SCF or H_2O_2: these data explain why H_2O_2 mimics the effect of this cytokine. In particular, the experiments suggested that PKB, PLCγ and the Src TK family, including Syk, in this order are involved in the glucose transport signal transduction, while AMPK, MAPK p38 and ERK aren't.

Induction of DNA oxidative damage, apoptosis and radiosensibilization in mouse neuroblastoma cells over-expressing spermine oxidase isoforms

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The flavoenzyme spermine oxidase (SMO) is an amino oxidase (AO) able to specifically oxidize spermine (SPM) to spermidine, 3-amino propanal and H_2O_2. By considering the role of polyamines, H_2O_2, and aldehydes in the cellular physiopathology, we analyzed the consequences of the over-expression of some cloned SMO isoforms in N18TG2 cells. Stable transfection of mSMOα and mSMOμ V5-tagged resulted in a five times increase in SMO activity. mSMOα localized in the cytosol and mSMOμ in nuclear and cytoplasmic compartments. Accordingly to the expected AO activity, the transfected cells showed a significant decrease of SPM and an increase of putrescine. In the nuclear extracts, only mSMOμ caused a significant decrease of SPM, while putrescine was higher for both active isoforms. The over-expression of SMO isoforms induced oxidative DNA damage, as measured in term of 8-oxo-7, 8-dihydroguanine formation, either alone or, in a higher extent, when associated with radiation exposure. The damage was correlated with the induction of apoptosis. These results indicate SMO as a possible modulator of the intracellular oxidoreductive status, as well as a sensitizer for oxidative treatments. In particular, dealing with the imbalance tissue specific SMO activity, they could indicate a new direction to tailor chemotherapy-associated radiotherapy, improving dose-rate protocol and allowing the modulation of deleterious side effects on healthy tissue.

ROS and PKC: Cell messengers able to trigger neuroblastoma apoptosis

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Reactive oxygen species (ROS) are mediators of cell growth and potential oncogenic transformation, and have been shown to play a role of "second messenger molecules" in the preservation of tissue homeostasis, namely apoptosis, cell cycle arrest and senescence. A crucial role in cell responses is played by Protein kinase C (PKC), a family of isoenzymes which can be activated or inactivated by ROS. Neuroblastoma produces oxygen intermediates and L-Buthionine-S,R-sulfoximine (BSO), a glutathione depleting agent commonly used in its clinical treatment, induces cell death. Our study provides evidence that ROS production, induced by BSO, is able to trigger mitochondrial apoptosis in neuroblastoma cells. This process of oxidative death is mediated by the activation of PKCδ which translocates to mitochondria and might be also involved in the production of ROS; moreover, early interaction between epsilon PKC and pro-apoptotic mitochondrial protein, Bax, has been observed and totally abrogated both by inactivation of δ isoenzyme and by antioxidant treatment. Future studies will be addressed to modify the expression and the activity of PKC isoenzymes in order to increase the efficacy of chemotherapy for neuroblastoma treatment.

Grants from University of Genoa, COFIN 2004 (#200468558_002), FIRB 2001 (#RBAU01JBH8_003), Italian Ministry of Health and Compagnia S. Paolo.

Determination of antioxidant activity of quercin, rutin and kaempferol in sorbitol-treatted human K562 cells

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Flavonoids represent a large group of naturally occurring polyphenols with a wide range of chemical structures and pharmacological activities. Three flavonols, aglycone quercetin (QUE), and glycone rutin (RUT; QUE-3-o-rutinoside), and aglycone Kaempferol (KAE), have attracted attention several years ago and they are being still studied very intensively because of their potential in the prevention of cardiovascular disease and cancer in human. The incubation time after sorbitol-treatment is a critical parameter; in fact, the typical ladder of apoptosis in agarose gel of cells with 1 M sorbitol showed DNA fragmentation not before 60 min. In the present study, we investigated protective effect of these flavonoids against sorbitol-induced apoptosis in human myelogenous leukaemia cells (K562) using DNA fragmentation assay To examine this possibility in cellular system, this study evaluated the capacities of the compounds to function as antioxidant in inhibiting apoptosis induced by sorbitol. K562, were pre-incubated with QUE, RUT and KAE (10ìM each for 2h) to allow for uptake and then the cell line were treated with 1 M sorbitol. Afterwards, cells were subjected to agarose gel electrophoresis to assess the DNA fragmentation. These compounds protected sorbitol-induced apoptosis, suggesting that the production of ROSs is involved in the mechanism. Furthermore, we reported that QUE, and RUT, and KAE were able to induce apoptosis in the K562 cells by dose-and-time dependent. This cell line, treated with different concentrations of QUE, RUT and KAE at different times showed DNA fragmentation. In conclusion, the antioxidant activity of three flavonoids is able to inhibit sorbitol-induced apoptosis in K562 cells.

Influence of curcumin and rosmarinic acid on growth of three different human cell lines

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Polyphenols are considered beneficial because of their potential protective role in the pathogenesis of multiple diseases associated to oxidative stress such as cancer and heart disease. However, many of this effects may depend on the concentration of the polyphenols utilized since high doses of some phenolic compounds may be prooxidant and negatively affect cell growth and vialibility. In this study, we investigated effect of curcumin and rosmarinic acid on cell viability in three different cell lines: erythroleukemia (K562), papillary (NPA), and anaplastic (ARO) thyroid cancers Curcumin was able to induce apoptosis in a concentration- and time dependent manner in all cell lines, while rosmarinic acid was effective on this process. only on ARO cells. Furthermore, rosmarinic acid and curcumin are two compounds with similar molecular structure, suggesting that they possess comparable chemical properties particularly in term of antioxidant activity. To examine this possibility in cellular system, this study evaluated the capacities of both compounds to function as antioxidant in inhibiting apoptosis induced by sorbitol. K562, NPA and ARO cells were pre-incubated with 25 ìM rosmarinic acid to allow for uptake and then the cell lines were treated with 1 M sorbitol. Afterwards, cells were subjected to agarose gel electrophoresis to assess the DNA fragmentation. Rosmarinic acid inhibited apoptosis induced by sorbitol. In conclusion, the antioxidant activity of rosmarinic acid is able to inhibit sorbitol-induced apoptosis.

Recruitment of glutathione into the nucleus in early phases of fibroblast proliferation

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Reduced glutathione (GSH) is a low molecular weight thiol and the most abundant no enzymatic antioxidant in the cell. It maintains cellular thiol/disulfide redox state and has a central place in the control of vital cell processes including cell proliferation (1). The peak of total GSH in 3T3 fibroblasts coincides with the peak of telomerase activity at 24h in culture and precedes the exponential phase of cell growth (2). Growing evidence show the importance of GSH compartimentation (3), and its role in numerous processes that occur in the nucleus. Therefore, we have followed the changes in the GSH distribution throughout the cell cycle. The GSH localization was studied by fluorescence determination of its CMFDA (5-chloromethylfluorecein diacetate) conjugate using confocal microscopy, and cell cycle by flow cytometry and protein expression of ID2 and p107 (western blotting). The nucleus/cytosol CMFDA fluorescence ratio reached a maximal mean value of 4.2 ± 0.8 six hours after cell plating. A ratio higher than 2 was maintained during exponential cell growth (at 24h, 41 ± 2% of the cells in S+M/G). At 5 days, as cells reach confluence and enter quiescence (81 ± 8 % of cells in G/G), the nucleus/cytosol ratio decreased to values close to one. We believe that the start of active proliferation of 3T3 fibroblasts requires a reduced environment in the nucleus and that the level of GSH compartimentation regulates the rhythm of the cell cycle.

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Redox status and DNA damage in healthy elderly individuals and relationship with GSTM1 genotype: Probin nutraceutical interventions

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The benefit of supplemental antioxidants in aging and chrnonic diseases is still under scrutiny. Fifty-four elderly patients were randomly divided in two matched groups. Group A was given a GMP-, ISO-9001-certified fermented papaya preparation (FPP, Osato Research Institute, Gifu, Japan) 9g/day by mouth while group B received a placebo. A cross-over design (3-month supplementation/6-week washout) was applied. Routine parameters, redox status and 8-OHdG in circulating leukocyte DNA were checked monthly. Genetic susceptibility studies (GSTM1 polymorphism analysis) were performed. GSTM1 genotype was null (-) in 40% and 46% of group A and B, respectively. GSTM1 (-) smokers had a significantly higher level of plasma DNA adducts and lymphocyte level of 8OHdG than GSTM1 (+) ($p < 0.01$). There was a weak correlation between cigarette/day and DNA adduct ($r = 0.61$, $p < 0.05$) which correlated with antioxidants concentrations but only in GSTM1 (-) smokers ($p < 0.01$). FPP-supplemented group showed a significantly improved antioxidant protection ($p < 0.01$ vs B) but only in GSTM1 (-) subgroup. Such protection was independent by other plasma antioxidants whose concentration didn't change. FPP treatment significantly improved plasma DNA adduct, irrespective of GSTM1 genotype ($p < 0.05$ vs placebo) while only GSTM1 (-) subgroup improved lymphocyte 8OHdG ($p < 0.01$) after FPP-supplementation. Such data show that FPP is a promising nutraceutical improving antioxidant defense in elderly patients even without any overt antioxidant deficiency and this may help explaining some contradictory or inconclusive results of interventional studies while paving the way to a science-based age-management.

Antioxidant action of *vitis vinifera* seeds and leave extracts

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The tissue integrity depends on the balance between production and degradation of the oxidants. Endogenous antioxidant defence may be insufficient to prevent oxidative damage and a corrected ingestion of antioxidant with diet may be necessary. The antioxidant power of a diet is not only its capacity to protect against oxidant species, but also to maintain constant the physiological production of oxygen reactive species and to improve antioxidant defence system. The aim of this study was to investigate the antioxidant activity of dry natural extract of Vitis Vinifera seeds and leaves (Giuliani S.p.A. Patent EP1328268B1) characterised by high amount of polyphenols (70%), in particular rich in catequin (7.67%) and quercetin (1.58%). We tested the extract (6; 12; 24 and 48mg/ml) scavenger activity against $^{\bullet}OH$, its ability to prevent oxidative damage induced by Cu^{2+} towards lipids using plasma as peroxidable substrate and its antiradical efficiency ($DPPH^{\bullet}$). The extract showed a scavenger activity against $^{\bullet}OH$ at 6 mg/ml while higher doses had not effect, the lipid peroxidation prevention was evident at doses 6 and 12 mg/ml. The antiradical efficiency of this extract could be classified as low. Based on our results the extract of Vitis Vinifera seeds and leaves showed antioxidant properties.

Oxidative stress and antioxidant status in patients on long-term home parental nutrition

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Aim: Parenteral nutrition could represents a cause of oxidative stress. Aim of this study was to evaluate oxidative stress and antioxidant status in patients(pts) with intestinal failure on long term home parenteral nutrition(HPN). Methods: 41 pts(M:16;F:25), mean age 61.05 ± 14.4 were studied. HPN median duration: 2636 d (172-5499). HPN regimen: n infusions/wk: 5.7 ± 1.15; VitE/PUFA: 0.52 ± 0.32mg/g/d. Se, vit A, C, E were supplemented according to recommended daily allowances (RDA) in HPN. 58 blood donors were considered as control. Plasma Malondialdehyde(P-MDA) and hydroxynonenale(P-HNE) as indexes of oxidative stress,and plasma selenium(P-Se), ascorbic acid(P-AA), dehydroascorbic acid (P-DHA), α-tocoferol, retinol, red blood cell-Glutathione Peroxidase (RBC-GSHPxSe) and glutathione (RBC-GSH) as indexes of antioxidant status were evaluated. Results are expressed as mean ± sd; Student t test for unpaired data between HPN and control group was used. *Results:* HPN pts vs controls: P-Se 66.2 ± 18.1 vs 81.1 ± 14 mg/L ($p<.0001$), P-AA7.2 ± 3.9 vs 8.4 ± 2.1 mg/L ($p < .05$) and P-α-tocoferol 8.4 ± 3.8 vs 11.8 ± 3.3mg/ml ($p <. 0001$), RBC-GSH 3.83 ± 0.8 vs 5.58 ± 1 mmol/gHb ($p <. 0001$), are lower in HPN pts; P-DHA 0.11 ± 0.19 mg/L ($p < .003$), P-MDA 4.74 ± 1.3 vs 3.01 ± 0.4 UF ($p < .0001$), P-HNE 8.11 ± 2.8 UF ($p < .0001$) are higher in HPN pts. *Conclusions:* Antioxidant system is compromised in pts in HPN. High DHA, MDA and HNE levels evidence oxidative stress. The replacement of micronutrients according to RDA was inadeguate to compensate for increased requirements.

Nitric oxide-induced tumor specific porphyrin fluorescence *in vitro*

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In photodynamic therapy (PDT) using aminolevulinic acid (ALA), cancer tissue accumulates protoporphyrin-IX (PpIX), which fluoresces and shows cytotoxicity under the excitation light. The decreased activity of an enzyme in heme biosynthesis, ferrochelatase, is suggested to be responsible for this phenomenon. Since ferrochelatase has a NO-labile active site, [2Fe-2S] cluster, the cancer-derived NO may decrease its activity, resulting in the fluorescence. To clarify it, we examined the presence of iNOS and intracellular NO concentration in cultured cells. We examined the effect of exogenous NO on the fluorescence intensities and ferrochelatase activities in cultured cells. *Methods*: MKN-45, derived from human gastric cancer, and RGM-1, from rat normal gastric mucosa, were immunostained with anti-iNOS antibodies, and their each intracellular NO concentration was examined with a NO- indicating fluorescent dye. The cells were cultured with ALA in order to measure their fluorescence intensities sequentially. The effect of a NO donor, S-nitroso-N-acetylpenicillamine (SNAP), on cellular fluorescence was also investigated. *Results*: Both iNOS expression and intracellular NO concentration of MKN-45 are significantly stronger than those of RGM-1. The fluorescence intensities of MKN-45 were significantly higher than those of RGM-1 60 min after the ALA treatment. They showed significant increase when co-treated with SNAP. *Conclusion*: This is the first report to investigate the role of NO on cancer specific porphyrin fluorescence. Since SNAP treatment increased both cellular porphyrin fluorescence and the amount of PpIX, we concluded that NO decreased the ferrochelatase activity. We proposed that NO from iNOS in cancer tissue caused the tumor-specific porphyrin fluorescence in ALA-PDT.

Effects of dietary selenium and α-tocopherol supplementations on in utero methylmercury neurotoxicity in mouse

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In utero toxicity of methylmercury (MeHg), thought to involve oxidative stress, affects perinatal brain development and may result in long-lasting neurobehavioral deficits. Two dietary factors, selenium and ω-3 fatty acids, which are unusually abundant in the traditional diet of the Inuit of Nunavik, might reduce perinatal developmental toxicity of MeHg in this exposed population. Mice exposed *in utero* to MeHg were used to assess the effects of diets enriched in α-tocopherol, selenium (Se) and/or n-3 PUFAs (ω3), against MeHg-mediated perinatal toxicity. Se- or α-tocopherol-enriched semi-purified diets reduced brain MeHg and mortality of newborns. The combination of Se and α-tocopherol, however, showed interactive toxicity *per se*. α-Tocopherol- but not Se-enriched diet suppressed offspring's motor coordination deficits associated with MeHg exposure. Supplementation of a low-Se/low-ω3 diet with both Se and ω3, but not each dietary factor alone, completely suppressed MeHg-mediated offspring mortality. Of note, the diet enriched in ω3 only was developmentally toxic *per se*, an effect suppressed by Se. These results support the hypothesis that elevated dietary intake of both Se and ω3 may reduce the developmental toxicity of MeHg, while Se-deficiency may enhance *in utero* MeHg toxicity, especially if combined with high intake of n-3 PUFAs.

Supported by Hydro-Québec/FRSQ, Health Canada and the Northern Contaminant Program, Canada.

Methods used in Europe to kill fish: Effects on the resource of α-tocopherol in Anguilla anguilla

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The welfare of fish has become one of the elements determining the total quality of the product. Ethically correct killing is part of the animal welfare concept and this final stage of life is the most critical both as regards the fish's suffering and the quality of the fish products included the antioxidants resource. As known, each fish species has characteristic tocopherol levels in its tissue, which, since fish are unable to synthesize the vitamin, are especially related to diet. The size or the age of the fish may also influence the tocopherol concentration and this concentration determine the oxidative stability of food. We reviewed the different methods of fish killing allowed in Europe and examined by HPLC in the finn chest strap cutting and CO_2 insufflation Anguilla anguilla muscle, the α-tocopherol antioxidative resource. Both killing methods implicated oxidative stress for Anguilla anguilla as proved by α-tocopherol muscle amount detected. Furthermore, we found a relation between fish size and amount of α-tocopherol and this resource, at each body size, is lower in CO_2 insufflation Anguilla anguilla killed. Our findings can be of practical relevance in the improvement of quality of this specie currently employed for human feeding and, in general for fish "shelf life".

Red wine and ethanol increase aromatase transcription in rat hippocampus

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Chronic ethanol consumption leads to oxidative damage in several central nervous system areas, resulting in an impairment of brain functions. The hippocampal formation, involved in memory and learning, is particularly sensitive to this kind of injury. It has been shown that estrogens have neuroprotective properties, and that the expression of aromatase, the estrogen producing enzyme, increases in neurons or astrocytes after neurotoxic insults. Although it may seem controversial, some alcoholic beverages have been linked to neuroprotection in the rat, although the mechanisms by which this occurs are largely unknown. We tested the effect of red wine or 13% ethanol solution consumption on the expression of aromatase in the hippocampal formation of the rat. Beverages were supplied to the animals as the only drinking source and a water control group was included. After 8 weeks of treatment, the animals were euthanised and the hippocampus dissected. Aromatase transcription was determined by RT-PCR. Aromatase transcript levels were increased by ethanol (to 158 ± 7% of control; $n = 4$) but significantly more so by red wine treatment (to 180 ± 9% of control; $n = 4$; $p < 0.05$). If the increase in aromatase expression induced by ethanol occurs as a response to the putative neurotoxic insult of this alcohol, the effect of red wine seems to indicate more than that and may corroborate the neuroprotective ability attributed to this particular beverage.

This work was supported by FCT (POCTI, Feder and Programa Comunitário de Apoio).

Modulation of aromatase activity by flavonoid compounds

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Estrogens are produced through the aromatisation of androgens to estrogens catalysed by aromatase. Besides their functions in reproduction, these hormones are involved in the regulation of physiological phenomena such as regulation of body weight and fat distribution, maintenance of bone mass and preservation of cognitive skills. Estrogens are also involved in the development of estrogen-dependent cancers. Polyphenols, abundant in vegetables, are food components known to modulate biochemical processes. For this reason, we decided to test the effect of daidzein, genistein, kampferol and naringenin in the activity and expression of aromatase. Choriocarcinoma derived JAR cells, that express high levels of aromatase, were preincubated with different concentrations of compounds for 2 h and aromatase activity was determined by tritiated water release assay, by incubating cells with test compounds and ^{3}H-androstenedione for 1 h. Genistein, kaempferol and naringenin dose-dependently inhibited aromatase, naringenin being the most potent [IC_{50} = 7.5 (3.2-17.5) μM]. Daidzein had no effect on aromatase activity. The effect on aromatase expression was also assessed by western blotting and RT-PCR after treatment of the cells with 100 μM of the compounds for 24 h, although no effect was observed. The results show that these food components are able to inhibit aromatase and that this occurs independently of aromatase synthesis. The inhibition of aromatase by polyphenolic compounds present in the diet may mean that the ingestion of certain foodstuffs potentially decreases estrogen synthesis what may be of interest in the management of estrogen-dependent diseases.

This work was supported by FCT (POCTI, Feder and Programa Comunitário de Apoio).

Modulation of vascular smooth muscle cells by polyphenols

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Angiogenesis, the formation of new blood vessels from pre-existing ones, is a complex process that comprises extracellular matrix degradation, endothelial cell proliferation and migration, basement membrane formation and adhesion of support cells such as smooth muscle cells (SMC). Angiogenesis is crucial for the development of cardiovascular diseases and cancer, presenting high mortality and morbidity rates in the western world. Polyphenols are naturally occurring compounds present in fruits and vegetables that are used in human diet. Several studies indicate the role of these compounds as anti-oxidants, anti-inflammatory and anti-cancer agents. Recent studies also indicate an anti-angiogenic role of these compounds. However, their exact role in SMC has not been clearly established. The aim of the present study was to evaluate the role of three distinct polyphenols in SMC behaviour. Viability, proliferation, apoptosis, migration and invasion rates were assessed in fetal aortic SMC cultures after incubation with xanthohumol (Xl), resveratrol (Res), epigallocatechin gallate (EGCG) or vehicles. Polyphenols led to a decrease in cell proliferation, whereas apoptosis was increased by every compound, as evaluated by TUNEL assay. Cell migration was also affected by these compounds. In addition, matrigel invasion was abrogated by the presence of EGCG. These findings suggest that besides affecting endothelia, polyphenols also play a role in decreasing survival, proliferation and invasion of SMC, a process that is required in the late steps of angiogenesis. Angiogenesis is essential for certain highly incident diseases development. The identification of diet compounds playing protective roles against these pathologies, render them putative agents for preventive strategies.

Aggregation of mitochondria and lysosomal rupture induced by β-amyloid in PC12 cells

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One of the principal pathological hallmarks of Alzheimer's disease (AD) is the presence of senile plaques in vulnerable brain regions. These plaques consist of a dense core of an aggregated peptide, amyloid β-peptide (Aβ). The 42 amino acid version is neurotoxic, inducing dendritic injury, sinaptic lose and neuronal death. Aβ 1-42 might induce mitochondrial injury and formation of free radicals. The aim of this work was to explore apoptosis-induce by Aβ peptide and to analysed how mitochondria and lysosomes are affected by this peptide. For this purpose, we compared the effect of the neurotoxic peptide (1-42) and the non toxic peptide (42-1) on PC12 cells. The treatment with a neurotoxic Aβn1-42 led to a significant increase in apoptotic cells and necrotic cells. We have shown that Aβ peptide caused mitochondrial aggregation, compared with control cells. Furthermore, we noted a lower fluorescence intensity of lysosomes in the cells, indicating a decrease of lysosomes in cells treated with Aβn1-42 ,probably due to lysosomal rupture. Our results indicate that the mitochondria aggregation and the lysosomes damage are involved in the toxicity of Aβ peptide and Aβ toxicity is induce by means of mitochondrial impairment. To our knowledge this is the first report of an induction of mitochondrial aggregation and lysosomal rupture due to Aβ.

Effect of polyphenol rich alcoholic beverages on rat hepatic alkaline phosphatase

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Mammals alkaline phosphatase (ALP) represents a family with 4 ALP isoenzymes. Tissue-nonspecific (tn)ALP is involved in bone mineralization and in regulation of several membrane transport systems. TnALP and intestinal (int)ALP are expressed in rat liver. Oxidative stress is known to interfere with ALP activity and expression. Red wine and beer components (such as ethanol) are expected to increase oxidative stress whereas others (polyphenols) are known to protect against it. On the other hand, these beverages directly modify ALP activity. This work aim was to investigate a) the effect of 8 weeks consumption of red wine (RW), blond (Blond) or black (Black) beer on rat hepatic ALP and catalase (CAT) activities and b) what ALP isoenzyme will be affected by these treatments. Control rats drank water (Wat), ethanol (Eth) 13% or 5.6% (RW and beer ethanol %, respectively). ALP was determined at pH=10.4 with p-nitrophenylphosphate as substrate (levamisole used as tnALP inhibitor) and CAT was determined as described by Aebi 1984, both in liver homogenate (n=4-5). ALP and CAT activities varied inversely. CAT/ALP values for Wat, Eth 5.6%, Eth 13%, Blond, Black and Rw groups were: 0.186, 0.320, 0.558, 0.250, 0.221 and 0.310, respectively. TnALP inhibition by levamisole was not identical for the 6 groups of rats. Eth 13% versus Wat seems to reduce intALP expression. RW versus Eth 13% seems to increase both ALP isoenzymes expression. Black versus Eth 5.6% seems to increase tnALP. Hepatic ALP and CAT activities, and hepatic ALP isoenzymes expression are modulated by alcoholic polyphenols rich beverages: polyphenolic and ethanol content and tecidular oxidative stress caused by them are involved in that modulation.

Support: FCT (POCTI, Feder and Programa Comunitário de Apoio) and iBeSa.

Kinetic study of the quenching reaction of singlet oxygen by flavonoids in ethanol solution: Can flavonoids protect the oxidative damage in foods and plants by quenching singlet oxygen?

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The quenching rate of singlet oxygen ($^1O_2$) by seven kinds of flavonoids (flavone, flavonol, chrysin, apigenin, rutin, quercetin, and myricetin) with 2,3-double bond has been measured spectrophotometrically in ethanol at 35°C. The overall rate constants, k_Q (k_q + k_r, physical quenching + chemical reaction), increased as the number of OH groups substituted to the flavone skeleton increases. The existence of catechol or pyrogallol structure in B-ring is essential for the $^1O_2$ quenching of flavonoids. The log k_Q was found to correlate with their peak oxidation potentials, E_P; the flavonoids, which have smaller E_P values, show higher reactivities. Similarly, log k_Q values of flavonoids correlate with the energy level of the highest occupied molecular orbital (E_{HOMO}) calculated by PM3 MO method and the longest-wavelength ππ* excitation energy (E_{ex}). The contribution of the chemical reaction (k_r) was found to be negligible in these flavonoids. The k_Q values of rutin, quercetin, and myricetin [(1.21~5.12) x 10^8 $M^{-1}s^{-1}$] were found to be larger than those of lipids, amino acids, and DNA. The result suggests that these flavonoids may contribute to the protection of oxidative damage in foods and plants, by quenching $^1O_2$.

S. Nagai, K. Ohara, and K. Mukai, *J. Phys. Chem. B*, **109**, 4234-4240 (2005)

Chelation of copper by three histidine residues of amyloid-β peptide inhibits the redox activity of copper

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Accumulation of amyloid beta peptide (Aβ) in the form of senile plaques is a pathological hallmark of the Alzheimer's Disease (AD). It has been reported that zinc, iron and copper are concentrated in the senile plaques. Copper catalyzes Fenton reaction, which leads to the oxidative stress. We have observed that Cu(II)-catalyzed hydroxynonenal formation from phospholipid hydroperoxides in the presence of reductant was inhibited by the addition of Aβ. Copper binding modes of Aβ have been documented. We have focused on the redox activities of Cu(II) bound to Aβ. Ascorbate oxidase activity and the intensity of ascobate radicals catalyzed by Cu(II) was decreased in the presence of Aβ. Ascorbate oxidase activity was strongly inhibited in the presence of Aβ1-16 and Aβ1-42 (< 80%), whereas slighty inhibited in the presence of Aβ1-12 and Aβ35-42(< 20%). The results are compatible with the effects of Aβ upon the intensity of ascorbate radicals formed in the ascorbate oxidase reaction by Cu(II). The present results indicate that chelation of Cu(II) by three histidine residues, His6, His13 and His14, inhibits the redox activity of Cu(II).

Effect of coffee drinking on platelet: Phenols incorporation and inhibition of aggregation

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Coffee is a widely consumed beverage, containing a multitude of substances, many of which potentially bioactive. Although the principal physiologic effects of coffee are attributed to caffeine, coffee is also an extremely rich source of chlorogenic acids, an important class of bioactive dietary phenols. Aim of this study was to establish if polyphenols from coffee (i) are incorporated in platelet and (2) exert an antiaggregatory effect. Ten healthy subjects drank 200 ml of filtered coffee (60 g/L) in fasting conditions. Platelet-rich plasma was separated from blood collected before (time 0), and 30 and 60 min after coffee drinking. After coffee drinking phenolic acids concentration (vanillic, caffeic, p-coumaric ferulic and isoferulic) increased significantly in platelets ($p < 0.05$ or $p < 0.01$ depending on phenolic acid; n=10). Coffee drinking inhibited platelet aggregation induced by 0.5 μM arachidonic acid ($p = 0.04$; $n = 10$) and by collagen at the concentration of 3.3μg/ml ($p = 0.05$; $n = 6$); while, it did not inhibit platelet aggregation when 2μM ADP was used as agonist. Coffee drinking also inhibited collagen-induced TXB2 formation ($p = 0.04$; $n = 6$). It has been reported that caffeine (the principal bioactive molecule in coffee) did not inhibit collagen-induced platelet aggregation. For this reason, we hypothesized that phenolic acids incorporated into platelets could be responsible for the observed antiaggregatory effect of coffee and that they could act through the decrease of platelet TXB2 formation, via the inhibition of COX.

This research was supported by ISIC-PEC

Mitochondrial function and mitochondria-induced apoptosis in an over-stimulated rat ovarian cycle

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Female rats were treated with FSH (40 IU/kg) in the first and second diestrus day (D1 and D2) and with LH (40 IU/kg) in the proestrus (P) day to synchronize and maximize ovarian changes. Follicle area increased by 50 % from D1 to P and the estrus (E) phase showed multiple corpora lutea and massive apoptosis. Increased oxygen uptakes (42-102%) were determined in ovary slices and in isolated mitochondria in active state 3 along the proliferation phase (D1-D2-P) that returned to initial values in the E phase. Mitochondrial content and the electron transfer activities of complexes I and IV were also maximal in the P phase (20-79% higher than in D1). Production of NO by mtNOS, biochemically determined, and the mtNOS functional activity in regulating state 3 oxygen uptake were also maximal at P and 79-88% higher than at D1. The moderately increased rate of NO in the proliferative phase is associated with mitochondrial biogenesis whereas the high rate of NO generation by mtNOS at phase P appears to trigger mitochondria-dependent apoptosis. The calculated fraction of ovary mitochondria in state 3 was at a minimal value at the P phase. Mitochondrial oxidative damage, with increased TBARS and protein carbonyls, indicate progressive mitochondrial dysfunction between phases P and E. The roles of mitochondria as ATP-provider, as a source of NO to signal for mitochondrial proliferation and mitochondria-dependent apoptosis, and as a source of O_2^- and H_2O_2 appear well adapted to serve the proliferation-apoptosis sequence of the ovarian cycle.

Effect of tea, wine and beer on ecto-alkaline phosphatase activity from human osteoblat cells (Saos-2)

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Alkaline phosphatase (ALP) is essential for bone mineralization and contributes to vascular calcification. Red wine, beer and tea (polyphenol rich beverages) consumption has been associated with protection against osteoporosis and cardiovascular diseases. Our aim was to investigate the effect of these beverages on ecto-ALP activity of human osteoblats (Saos-2) and compare it with our previous results on human adult aortic smooth muscle cells (AALTR). Ecto-ALP activity in intact Saos-2 was determined at pH=7.8 with p-nitrophenylphosphate as substrate. Results expressed ecto-ALP activity recovered as % of control, n = 5-9, $p < 0.05$. Saos-2 ecto-ALP activity was tissue-nonspecific ALP as demonstrated by levamisole inhibition (1.80 ± 0.39 of recovered activity). Tea showed a strong inhibitory effect on ecto-ALP: 9.23 ± 2.71% with green tea and 15.26 ± 3.56% with black tea. Wine did also inhibit ecto-ALP: 25.89 ± 3.78% with red wine (higher polyphenolic content) and 75.62 ± 2.32% with white wine. Beer had an intermediary effect (53.55 ± 6.28% - 82.49 ± 3.82%). There was no difference between the results obtained with beer and wine and the corresponding dealcoholized beverages. Ethanol in water solutions (1.0-15.0%) had no effect on enzyme activity. So, the effect of the tested beverages on Saos-2 ecto-ALP activity were most probably due to their polyphenolic compounds. These same beverages had a higher inhibitory effect on ecto-ALP of AALTR cells. The differential effects of the beverages over the activity of osteoblastic and vascular cells ecto-ALP do not explain the presumed protective effect of the beverages against osteoporosis but may be involved in cardiovascular protection through vascular calcification prevention.

Support: FCT (Programa Comunitário de Apoio e FEDER) and iBeSa.

The effect of vitamin E supplementation on immune modulation in normal human volunteers

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Our study investigated the effect of vitamin E supplementation, specifically tocotrienols and alpha-tocopherol on immune modulation in healthy, young subjects. The trial had a parallel, double-blind, randomised, controlled design and adhered to the Good Clinical Practice (GCP) guidelines. Volunteer pre-screening consisted of a blood screen, general physical examination and history taking and only volunteers who met the predetermined eligibility criteria were recruited. Nineteen males and 34 females aged between 20- and 50-years were given eight-week daily oral supplements of either placebo, 200 International Units (IU) of alpha-tocopherol or 200 milligrams (mg) of palm oil tocotrienol-rich fraction (TRF). Blood was taken from the volunteers on days 0, 28 and 56 and was used to measure plasma vitamin E levels and mitogen-stimulated T-cell culture to determine the types of cytokines produced. ELISA was used to measure interferon-gamma, interleukin-4 and IL-10 concentrations from the T-cell culture supernatants. Other immune parameters such as T-cells, B-cells and Natural Killer (NK) cells were also measured via flow cytometry. Statistical analyses used include one way analysis of variance (ANOVA) on the percentage changes in biochemical parameters and mixed or split-plot ANOVA (SPANOVA) on immune parameters. Immune modulation was determined via statistically significant mean differences between the experimental groups. SPANOVA was statistically significant for serum alpha-tocopherol and total vitamin E concentrations, with both alpha-tocopherol and TRF groups having higher means than the placebo group. SPANOVA results were largely not statistically significant for immune parameters, and thus we concluded that vitamin E supplementation did not affect immune modulation in healthy, young subjects in the absence of an immunogenic stimulation.

Antiradical activity of the methanolic extract from *Irvingia gabonensis* seeds

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The aim of this study was to evaluated the antioxidant activity of the methanolic extract from Irvingia gabonensis seeds. The content of the phenolic compounds of that extract was determined and the antiradical activity was evaluated using the free radical, 2,2-diphenyl-1-picryl-hydrazyl (DPPH$^{\bullet}$) as free radical. The anti radical activity was defined as the amount of extract necessary to decrease the initial DPPH$^{\bullet}$ concentration by 50% (efficient concentration). The methanolic extract from Irvingia gabonensis reacted slowly with the DPPH$^{\bullet}$ and reached the steady after 1 hour. That extract showed a higher antiradical activity with a EC50 of 0.098 mg of extract /g of DPPH$^{\bullet}$. The number of DPPH$^{\bullet}$ moles reduced by one mg of extract which is the inverse value of quantity of extract needed to reduced 100% of the DPPH$^{\bullet}$ was 5. The methanolic extract from Irvingia gabonensis was a strong radical scavenger indicating that active compounds of different polarity could be present in this plant.

Cell death in PC-12 cells induced by 3,4-dihydroxyphenylacetic acid and nitric oxide. The critical role of glutathione

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Parkinson's disease is one of the most prevalent age-associated neurodegenerative diseases. Although the precise mechanisms underlying the cell death in this disease are not clearly established, there are many studies suggesting that an excessive or inappropriate formation of •NO (or its derivatives) and an abnormal metabolism of dopamine may modulate cell events that are involved in the neurodegenerative processes. In the present study, we establish a pathway for cell death in PC-12 cells, a cell line often used as a neuronal model, involving •NO and 3,4-dihydroxyphenylacetic acid, (DOPAC) a metabolite of dopamine. The results show that DOPAC modulates •NO effects on PC-12 cells; whereas at high concentrations, cell viability is compromised, at low concentrations an increase in cell viability is apparent. The cell death induced by •NO involves caspase activation but when cells were co-incubated with DOPAC and •NO the occurrence of cell death independent of caspases activation was noticed. In the later case, a depletion of GSH content precedes cell death, pointing to its involvement in the combined effect of DOPAC and •NO on cell viability. The results suggest that •NO and DOPAC interact in PC-12 cells and for high concentrations may underlie cell death associated with neurodegenerative processes.

Supported by FCT: SFRH/BD/5428/2001 and POCTI/BCI/42365/01

Effects of zinc on angiotensin converting enzyme (ACE) activity

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Despite zinc ion (Zn) displays a fundamental role in the activity of several enzymes, it may also present inhibitory activities. In this study, we characterized Zn effect on ACE (EC 3.4.15.1) activity, using angiotensin I (AI) and bradykinin (BK) as substrates. Zn inhibited differentially AI (IC_{50} 6.7 ± 0.7 μM) and BK hydrolysis (IC_{50} > 2 mM). This inhibition was not dependent on the anion, since chloride, sulfate and acetate Zn salts presented similar inhibitory effects. EDTA (< 0.2 mM) reverted the Zn (0.1 mM)-mediated ACE inhibition when AI was used as substrate, but had no effect when BK was the substrate. However, concentrations higher than 0.2 mM EDTA inhibited ACE activity using both, AI or BK as substrate. The activities of AI and BK to bind Zn were measured as the inhibition of the fluorescence of the FURA-2-Zn complex. AI presented a Ki value about 113 μM, while BK did not present any activity. To confirm that AI hydrolysis inhibition was mediated by the sequestration of the substrate, ACE activity was determined using different Zn and Ang-I concentration. The obtained data suggest the participation of the complex AI-Zn, in the Zn-mediated ACE inhibition. This study alerts on the relevance of Zn on ACE activity, not just as constituent of the active site of the enzyme but also as a modulator of its activity.

Modulation of skin oxidative stress markers by environmental stressors: Differences between young and old

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Skin is the first barrier against outdoor environment pollutants such as ozone (O_3), cigarette smoke (CS) and ultraviolet radiation (UV). Environmental factors have been recognized to induce modifications of the morphological and biophysical properties of the skin. Epidemiological studies have indicated an association of skin aging with UV and CS exposure. Furthermore, O_3 induced stress responses in skin have been recently documented. The present study was targeted to investigate skin responses to environmental stressors in young and aged mice. We determined the effect of pollutants i.e. UV (0.3 MED), O_3 (0.25 ppm 6hr per day) or CS (60 mg/m3 6 hr per day) alone or in combination for 4 days in the skin of young (4-8 wk) and aged hairless mice SHK1 (72 wks). Proteins were extracted from whole skin and oxidative stress markers including 4HNE and HO-1 were measured by Western blot. Both HO-1 and 4HNE were induced after environmental stressor exposure. Old mice showed an increase in 4HNE protein adduct formation. Basal levels of HO-1 were higher in young control mice. Aged skin was more susceptible to environmental stressors induced HO-1 (25% increase by O_3 and 100% by UV). Furthermore, a significant induction of $p22^{phox}$ (but not $p47^{phox}$) and phosphorylated ERK was detected upon stressors exposure. These results were more evident in old skin, especially after O_3 exposure (2 fold increase). Finally, the oxidative stress/aging marker $p66^{shc}$ was also more expressed in young skin. These data suggest that environmental pollution can differentially affect cutaneous stress responses using different pathways depending on the stressor and on the age.

Adaptation to chronic intermittent hypoxia in the heart

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Obstructive sleep apnea (OSA) has emerged as an important public health problem since this condition is associated with increased risk for cardiovascular diseases. As OSA is characterized by episodic cycles of hypoxia and normoxia during sleep, we investigated effects of intermittent hypoxia (IH) on ischemia/reperfusion (I/R)-induced cardiac injury. C57BL/6 mice were subjected to episodic hypoxia (2 min cycles of 6% and 21% O_2) for 8 h/day for 1, 2 and 4 wks. After IH, isolated hearts were subjected to I/R. Consistent with OSA patients developing systemic hypertension, IH increased mean arterial blood pressure. IH for 1 or 2 wks significantly enhanced I/R-induced LDH leakage and infraction. Interestingly, however, enhanced I/R-induced cardiac damage was not seen in mice treated with 4 wks of IH, suggesting that the heart has adapted to chronic IH. 1 - 2 wks IH activated a novel stress responsive myocardial survival and repair factor GATA-4 without influencing its gene expression. Despite the enhanced basal activity of GATA-4, in mice subjected to 1 or 2 wks of IH, I/R caused inhibition of the GATA-4 activity, which might be responsible for exaggerated I/R- induced cardiac damage. In contrast, I/R did not inhibit GATA-4 activity in hearts from mice treated with 4 wks of IH. In these hearts, higher percentage of GATA-4 molecule is phosphorylated. Further, hearts subjected to 4 wks of IH had enhanced catalase activity in response to I/R. We proposed that the reversal of IH-mediated increase in cardiac damage is in part due to GATA-4 modification and enhancement of antioxidant defense mechanism.

Supported by NIH HL67340 & HL72844

Regulation of Bcl-x_L gene transcription by intermittent hypoxia

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The heart is subjected to oxidative stress during various pathophysiological conditions and the identifications of endogenous cardioprotective mechanisms may lead to therapeutic strategies against heart diseases. Recent studies have shown that short-term intermittent hypoxia (IH) can induce preconditioning-like events to protect the heart against oxidative stress. The objective of the present study is to identify regulators of IH-mediated cardioprotection. After C57BL/6 mice were subjected to 5 cycles of 2 min hypoxia (10% O_2) and 2 min normoxia (21% O_2), hearts were homogenized to isolate RNA and IH-inducible genes were identified by Microarray analyses. Anti-apoptotic bcl-xL mRNA levels were found to be increased in the hearts from mice subjected to IH. RT-PCR confirmed these results by showing that bcl-xL mRNA levels are transiently increased with a peak at 6 h after IH. We found that protein levels of Bcl-x_L was also upregulated. The increased gene transcription of Bcl-x_L may be due to the activation of protein acetylation, as IH increased acetylation of nuclear proteins. We also found that another form of lysine modification sumoylation was modulated by IH. Interestingly, modifications by SUMO-1 was increased, while those by SUMO-2/3 were decreased by IH. We propose that IH-induced oxidative modification of histone deacetylase may induce protein acetylation and promote gene transcription of Bcl-x_L in the mechanism of cardioprotection exerted by short-term IH.

Supported by NIH grants HL67340 and HL72844

Interactions of quercetin and rutin with Cu and Pb ions and their influence on free amino acids concentrations in rat tissues

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Toxic action of metals like Cu or Pb can be reduced by applies of diet enriched in substance able to chelate those metals but without any cytotoxic properties. Quercetin and rutin belong to the group of such compounds. The aim of our study was to determine the effect of quercetin or rutin administered together with lead or cooper on the level free amino acids in serum blood and in liver of experimental rats. The experiment was conduced 6 weeks on Wistar male rats, divided into 7 groups, each of 6 rats. Groups I was a control group, and those animals received redistilled water to drink. Groups II-IV of animals obtained in drinking water solution cooper chloride, $CuCl_2$ in amount of 300 mg/dm^3 in reduction to unalloyed metal and additionally group III 100 mg/dm^3 of quercetin, groups IV 100 mg/dm^3 of rutin. The V-VII groups obtained the sae that groups II-IV but they received instead of cooper plumbum nitrate, $PbNO_3$ in amount of 300 mg/dm^3 in reduction to unalloyed metal. Free amino acids were determined in liver and blood serum. Addition of the bioflavonoid to metal solution caused decrease of amino acids level in liver in comparison to group that obtained metal solution only. This decrease was more significant in case of rutin than of quercetin and in case of Pb ions than Cu ions. In blood serum amino acids level fluctuates were observed in dependence on examined group but also kind of amino acid and its function in organism.

The influence of chosen low molecular mass antioxidants on the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in rat's tissue

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The ascorbic acid (AA) is low molecular mass antioxidant that scavenges the aqueous ROS by very rapid electron transfer that inhibits lipid peroxidation. Zinc and copper are the elements that interact directly with superoxide dismutase and in that way protect the cells from reactive oxygen species. The present investigation was carried out to study the effect of AA plus elements - zinc and copper on the activities of antioxidant enzymes: superoxide dismutase (SOD) and glutathione peroxidase (GPx). The study was made on two-months-old Wistar male rats divided into 6 groups each of six rats, which received in drinking water - ascorbic acid (5 mg/g of body weight) alone/and with zinc and copper. The glutathione peroxidase (GPx) activity was determinated by Beauchamp and Fridovich method, superoxide dismutase (SOD) activity by Paglia and Valentine method. Tissue specific changes following elements exposure and responses to the treatment with AA were recorded in the parameters of antioxidant enzymes. In almost all tissues an increase of the activities of antioxidant enzymes have been noticed.

Cigarette smoke and UV light as environmental stressors to skin keratinocytes : Estimation of NO and $ONOO^-$ production

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Introduction: UV light and cigarette smoke are both considered as skin ageing promoters. The effect of cigarette smoke on skin is much less studied comparing to UV light while their synergistic effect is not well documented. *Methods*: Skin primary keratinocyte mice cultures were suspended in phosphate buffer (PBS pH 7.4). In defined environmental conditions they were submitted to UV light or cigarette smoke or to an association of them. The release of NO by the skin keratinocytes was estimated as ONOO- by lucigenin-enhanced chemiluminescence in the presence of H_2O_2. By using luminol-enhanced chemiluminescence the ONOO- production was evaluated. *Results*: The oxidative stress estimated in the UVB irradiated skin keratinocytes exhibited a much higher level as compared to non-irradiated keratinocytes (control). UV light release NO in relatively important quantities while UV + cigarette smoke less and cigarette smoke alone much less. UV light produce small relatively quantities of ONOO- while UV + cigarette smoke much more and cigarette smoke more considerable quantities than UV alone. *Discussion*: Several studies have documented increased oxidative stress values in aging. Our present studies suggest that NO and ONOO- are key factors and no by standards in aging process. It seems that the role of oxidative stress determines the dangerous ONOO- production where a synergistic effect of UV and cigarette smoke is obtained. Future research should establish whether free radicals initiate, maintain, challenge or worsen this process.

Pro-oxidant properties of some diimine-copper(II) complexes toward biomolecules involving ROS

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Although present in the active site of many proteins and enzymes performing key physiological roles, copper ions not properly coordinated in vivo can also participate on oxidative processes that lead to pathological conditions and degenerative diseases. On the other hand, many copper-based drugs have been recently investigated as potential antitumor agents, based on their oxidation-promoting activity. These metal complexes are usually capable of efficiently generate reactive oxygen species (ROS), during catalyzed oxidation of endogenous substrates, triggering oxidative stress. Some different copper(II) complexes, with tri- or tetradentate diimines containing indole, pyrazine, or pyridine groups, have been isolated and characterized by different techniques (UV/Vis, IR, EPR), and had their reactivity in oxidation processes investigated. These complexes were able to causing oxidative damage to albumine, in the presence as well as in the absence of hydrogen peroxide, as monitored by the carbonyl groups formed in the protein. They were also compared as ROS generators, by EPR spin-trapping measurements, showing significant pro-oxidant activity, modulated by the di-imine ligand. Previous studies by CD and UV/Visible spectroscopy indicated two possible sites for the interactions of these complexes with the protein, and that the damage caused is very dependent on the copper binding site.

Financial Support: FAPESP, CNPQ, and CAPES.

Role of oxidative and nitrosative stress in taurocholate-induced necrotizing pancreatitis

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Oxidative stress has been implicated in the development of acute pancreatitis. Aim: 1) To study the evolution of oxidative stress in acute pancreatitis, and its relatioship with nitrosative stress and the induction of pro-inflammatory genes; and 2) To assess the effect of preventing oxidative stress on nitrosative stress and on the induction of pro-inflammatory genes. In the present work, we have used the experimental model of necrotizing pancreatitis induced by 3.5% in rats. The treatment with oxypurinol –inhibitor of xanthine oxidase- and pentoxifylline –inhibitor of TNFα production- was use to diminish oxidative stress. Reduced (GSH) and oxidized (GSSG) pancreatic levels were measured. Protein nitration in pancreas was studied by western blotting. The expression of TNFα, iNOS and ICAM-1 was determined by RT-PCR. The regulation of these genes was studied by the chromatin immunoprecipitation assay. Glutathione oxidation and consequently oxidative stress were found at 3 h post induction and thereafter, whereas protein nitration, indicative of nitrosative stress, was found from 1 h postinduction. The up-regulation of pro-inflammatory genes was found at 3 h and thereafter. The induction of iNOS and ICAM was associated with NF-κB binding to their promoters, whereas NF-κB and SP1were involved in the induction of TNFα. Treatment with oxypurinol prevented glutathione oxidation and diminished nitrosative stress, but it did not change the expression of pro-inflammatory genes. Pentoxifylline prevented GSH depletion, and diminished both iNOS expresión and nitrosative stress, avoiding the up-regulation of TNFα and ICAM-1.

Adenosine A2a receptor activation attenuates the proteolytic mechanism of reperfusion damage in rat liver

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Ischemia-reperfusion injury (IRI) to the liver is a multifactorial process seen after surgical procedures in which vascular supply to the organ is temporarily abrogated. Studies on ischemic preconditioning showed that adenosine A2a receptor activation stimulates cellular mechanisms of protection against sustained irreversible ischemic injury. The aim of this study was to evaluate whether pharmacological preconditioning by adenosine A2a receptor agonist (CGS21680) treatment could attenuate the calpain-dependent injury in a rat model of liver IRI. IRI was produced in male Sprague-Dawley rats through a lobar selective inflow occlusion model. CGS21680 (10 μg/kg) was administered i.v. 5 min before ischemia. Necrosis was evaluated by ALT levels in plasma and by morphology. Calpain activity in cytosolic enriched fractions was measured by fluorometry and the proteolysis of talin was evaluated by Western Blotting. After 90 min of ischemia/1h of reperfusion, CGS21680 treatment reduced almost 50% the level of ALT with respect to the value observed in the vehicle treated rats. Pharmacological preconditioning was able also to reduce by 40% calpain activity after ischemia and reperfusion phases respect to vehicle group. Highly calpain-dependent talin demolition was observed at the end of re-flow period, in contrary CGS21680 preserved talin levels. These results indicate that pretreatment with CGS21680 attenuates the proteolytic-dependent mechanisms of early reperfusion damage in the liver.

NADPH oxidase-dependent thrombus growth

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Background. Platelet recruitment at site of vascular injury is determinant for thrombus growth. Platelet nitric oxide(NO) has an inhibitory effect on platelet recruitment but it is unclear if oxidative stress is implicated on its modulation. Aim of the present study is to evaluate the role of $gp91^{phox}$, the central core of NADPH oxidase, in platelet recruitment. *Methods*. In two male patients with hereditary deficiency of $gp91^{phox}$, the central core of NADPH oxidase, and in 10 male healthy subjects matched for age agonist-induced platelet aggregation and platelet recruitment were determined; in both groups agonist-induced platelet production of $O_2^{\cdot-}$ and NO was also measured. *Results*. Compared to controls, $gp91^{phox}$-deficient patients had almost complete suppression of platelet $O_2^{\cdot-}$ and a significant increase of platelet NO ($p < 0.005$). $gp91^{phox}$-deficient patients had normal agonist-induced platelet aggregation and thromboxane A_2 formation but significant reduction of platelet recruitment ($p < 0.005$). Incubation of platelets from $gp91^{phox}$-deficient patients with LNAME, an inhibitor of NO synthase, restored platelet recruitment; similar results were obtained in platelets treated with the cGMP inhibitor LY83583. *Discussion.* These findings indicate that an imbalance between oxidative stress and redox status has a crucial role in the process of platelet recruitment. Inhibition of $gp91^{phox}$ may represent a novel target of antiplatelet treatment.

Improvement of mitochondrial function by inhibition of the renin-angiotensin system (RAS) in spontaneously hypertensive rats (SHR)

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We have previously showed that RAS inhibition prevented the decay of mitochondrial function in kidneys of SHR, independently of blood pressure reduction. Here we studied whether a non-hypertensive dose of enalapril, an angiotensin converting enzyme inhibitor, could protect cardiac mitochondria from hypertension-related dysfunction. Three-month-old SHR received water containing enalapril (10 mg/kg/day, E) or no additions (S) for 5 months. Wistar-Kyoto rats (W) were normotensive controls. At the end of study blood pressure was higher in E and S relative to W. In S, heart/body weight ratio was higher than in E and W. Heart hypertrophy could result because of tissue remodeling associated to oxidant-activated matrix metalloprotease (MMP), since MMP activity was lower in E with respect to S and W. In S, mtNOS activity and eNOS expression and activity were lower compared to E and W. Mitochondrial membrane potential in E was lower than in S and W; and hydrogen peroxide production was higher in E and S compared to W. In S, Mn-SOD activity was higher than in E and W. NADH-dehydrogenase activity was lower in S and E than in W. In summary, in SHR enalapril protects from cardiac hypertrophy, and attenuates cardiac mitochondria dysfunction independently of blood pressure reduction. These results will be discussed considering the effects of enalapril in preventing oxidant production secondary to the inhibition of the RAS.
Supported by ANPCyT 01-08951 (Argentina).

Necessary and sufficient parameters of blood plasma for clinical characterization of oxidative stress in human organism

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A principal characteristic of living organisms is their capability to actively protect themselves against uncontrolled oxidation. Under the permanent influence of potentially noxious causes (UV sun radiation, atmospheric pollutants, some medical preparations, etc.), they maintain their integrity due to the effect of the antioxidative system of the organism, developed during course of phylogenesis, which regulates the blood level of antioxidants according to the demand. Which substances are to be determined minimally in the clinical routine for detection of dangerous deviations in symptom-free stages of illnesses and for control of the therapy effectiveness? Our experimental and clinical results showed that the more important are antiradical capacities of urate (UA) and ascorbate (ASC). The antiradical ability of proteins (ARAP) is not a feature of an antioxidative defense as assumed early, but more really a measure of oxidative damage to them. Summarized, we demonstrate that the most suitable for routine measurements are following components of an integral antiradical capacity of water soluble substances in blood plasma (ACW): ACW = UA + ASC + ARAP + AO, here AO is the sum of unidentified antioxidants (normally, under sober condition AO < 5% of ACW, the percentage can be bigger after consumption of fruits and vegetables). The usefulness and connections between the parameter will be demonstrated at examples of the metabolic (physical exercises) and environmental (UV whole body irradiation) oxidative stress.

Effect of antioxidants and inhibitors of NAD(P)H oxidase and tyrosine kinase activities on glucose uptake in a megakaryocytic cell line

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According to a newer view, deliberate reactive oxygen species (ROS) generation occurs not only in phagocytes but also in other cell types, in which ROS function in cell signalling and metabolism. We have recently studied the relationship between basal level of intracellular ROS and Glut1 activity in a human megakaryocytic cell line, B1647. Glut1 is the transporter isoform responsible for the basal glucose uptake in many cell types and is subjected to "acute" regulation by several metabolic and oxidative stresses. Since the evidence supporting the concept of ROS as signalling molecules is based on the observation that antioxidants and inhibitors of ROS-generating systems block specific physiological responses, we tested the effects of EUK-134, a synthetic SOD and catalase mimetic, on glucose uptake and intracellular ROS level, and we showed that ROS are important to maintain the activation of glucose transport mediated by Glut1. In order to confirm the observed effect of EUK-134 and to obtain information about which reactive species might be involved in glucose uptake, we tested the effect of several ROS scavengers. Results show that different species are involved in this activity. Data obtained in the presence of NAD(P)H inhibitors suggest that a possible ROS generation site could be this membrane-bound enzymatic complex, similar to the phagocytic one. The effects of tyrosine kinase inhibitors and antioxidants show the importance of phosphorylation process in the regulation of Glut1 activity and that a possible target of ROS as molecular signal are protein phosphatases, as reported in literature.

Workplace exposure to environmental tobacco smoke: Impact on risk factors for coronary heart disease and biomarkers of oxidative stress

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This study was designed to investigate the impact of workplace environmental tobacco smoke (ETS) exposure on risk factors associated with coronary heart disease and biomarkers of oxidative stress, and the potential moderation of these effects by antioxidant supplementation. Non-smoking bartenders, cocktail servers and casino workers in the Las Vegas and Reno, NV areas, not exposed to ETS in the home but in the workplace were recruited. Following initial baseline analyses of these risk factors, subjects were randomized into one of three antioxidant supplement groups. Group 1 received a placebo; Group 2 received low daily dosages of an antioxidant cocktail of approximate RDA levels 75 mg Vitamin C, 15 mg Vitamin E and 60 μg selenium; Group 3 received daily dosages approximating one-half the established upper limit for daily intake of these three antioxidants, 1000 mg Vitamin C, 500 mg Vitamin E and 200 μg selenium. Subjects returned every 6 months for up to two years for re-evaluation of their risk factors and biomarkers. 8-hydroxy-2'-deoxyguanosine, homocysteine, C-reactive protein, total antioxidant capacity, oxidized LDL, cholesterol, LDL cholesterol, HDL cholesterol, superoxide dismutase, glutathione peroxidase and cotinine were determined in these subjects.

This study was funded in part by NIEHS grant ESO9520.

The intake of a single portion of blood orange juice increases lymphocyte protection against oxidative-induced DNA damage: is it an effect of vitamin C?

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Epidemiological studies suggest that citrus fruit consumption is protective against several types of cancer. In particular, it is presumed that most of the protective effect is due to vitamin C, however, several studies have suggested that the frequency of citrus fruit consumption is more closely related to risk reduction than vitamin C intake. In the present study we assessed the effect of the consumption of a single portion of fresh blood orange juice (BOJ), water added with vitamin C (C-drink) or sugars (S-drink) on lymphocyte resistance to oxidative DNA damage. Seven healthy female volunteers were enrolled in a randomised repeated-measure design in which they had to assume each drink (300 ml BOJ or C-drink, both containing 150 mg vitamin C, and S-drink, containing the same amount of sugars) on three different occasions, at least 10 days apart. Plasma vitamin C concentration was evaluated by HPLC at baseline, every hour for 8 hours and 24 hours after consumption. The comet assay was used to measure lymphocyte DNA resistance to oxidative-induced damage at baseline, 3 and 24 hours after the drinks intake. Both BOJ and C-drink consumption determined a significant increase in plasma vitamin C concentration 1 hour after the intake, with a peak at two hours. S-drink intake did not affect plasma vitamin C concentrations. Lymphocyte DNA resistance to oxidative stress increased 3 hours after the intake of BOJ and remained high at 24 hours. On the contrary, no effect was seen after both C-drink and sugar-drink consumption. The present work gives further support to regular fruit consumption, instead of single antioxidant supplementation, in order to improve cell protection.

Proliferation and migration of human aortic smooth muscle cells induced by oxysterols

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Proliferation and migration of smooth muscle cells (SMCs) in the intima are key events in the arterial thickening leading to atherosclerosis and restenosis. Low density lipoproteins (LDL) undergoing oxidation especially within the arterial wall are postulated to play an important role in these events. Among the oxidized lipids detectable in oxidized LDL, cholesterol oxidation products (oxysterols) are increasingly considered of potential interest in atherogenesis. Since oxysterols are constantly present in the oxidized LDL as a mixture, we used a mixture of oxysterols representative of that found in plasma of hypercholestero-lemic individuals, to modulate the proliferative state and the migration potential of cultivated aortic human SMCs. In a concentration range of pathophysiological interest (7.5-10 μM) the oxysterol mixture induced a marked increase of human aortic SMCs proliferation, in particular after 48 h treatment. Such an effect was not concentration-dependent. Further, as regards the contribution of the individual components to the effect produced by the oxysterol mixture, the single oxysterols did not show any effect on SMCs. This evidence pointed out that a cooperative effect exists among the different oxysterols when they are given to cells as a mixture. As regards cell migration, the oxysterol mixture used at 10-20 μM induced a significant increase of SMCs chemotaxis. Experiments to identify the molecular mechanisms underlying the observed oxysterol-induced effects are in progress.

Dietary antioxidants and disease

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A diet rich in fruits and vegetables reduces the risk of cancer and other oxidative stress related diseases. The most likely hypothesis is that antioxidants from fruits and vegetables reduce oxidative stress. These antioxidants may cooperate in a network and a combination of different antioxidants may be needed to protect the cells against oxidative stress. Our group has developed both transgenic reporter mice models and chromatographic techniques in order to study the effects of antioxidant-rich diet on diseases. To test the antioxidant network hypothesis, we collected 7-d weighed dietary records in 61 adults and used data from a nationwide survey of 2672 Norwegian adults. The total intake of antioxidants was 17 mmol/d with β-carotene, α-tocopherol, and vitamin C contributing <10%. The intake of total antioxidants was significantly correlated with plasma lutein, zeaxanthin, and lycopene. These data agree with the hypothesis that dietary antioxidants other than the well-known antioxidants contribute to our antioxidant defense. In an another study we have assessed systemic oxidative stress in head and neck squamous cell carcinoma (HNSCC) patients by measuring endogenous antioxidant total glutathione (tGSH) in plasma. We found that tGSH was lower in HNSCC patients than controls, and the patients with low tGSH levels had lower survival rate.

Svilaas A, Sakhi AK et al. Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans. J Nutr. 2004;134, 562-7

Endothelial function in an animal model of type 2 diabetes: Dyslipidemia and lipoic acid

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Diabetes is a major risk factor for vascular diseases. Diabetes mellitus is also associated with an increased production of reactive oxygen species and a reduction in antioxidant defenses. This leads to oxidative stress, which is partly responsible for diabetic complications. Antioxidant therapy may be useful in preventing atherosclerosis. Alpha-lipoic acid (LA) is a multifunctional antioxidant and has been shown to have beneficial effects on polyneuropathy and on the parameters of oxidative stress in various tissues. This study was conducted to investigate the effects of LA on endothelial function in diabetic and hypercholesterolemic animal models. Carbohydrate and lipid metabolism, endothelial function, plasma malondialdehyde (MDA) and urinary 8-hydroxydeoxyguanosine (8-OHdG) were assessed in non-diabetic controls (Wistar rats), untreated Goto-Kakizaki (GK) diabetic and atherogenic diet (AD) fed GK rats (fed with atherogenic diet only, treated with alpha-lipoic acid and treated with vehicle, for 3 months). AD resulted in a 3-fold increase in both total and non-HDL serum cholesterol levels and in a 2-fold increase triglyceride levels while endothelial function was significantly reduced. MDA and 8-OHdG levels were higher in GK and GK hypercholes-terolemic groups and fully reverted with the antioxidant. Hypercholesterolemic GK diabetic rats showed significantly reduced endothelial function that was partially improved with LA. Furthermore, lipoic acid significantly reduced serum cholesterol levels, without lowering HDL cholesterol in hypercholesterolemic GK diabetic rats. Alpha-lipoic acid supplementation represents an achievable adjunct therapy to improve endothelial function and reduce oxidative stress, factors that are implicated in the pathogenesis of atherosclerosis in diabetes.

Antioxidant effects of α-lipoic acid in the diabetic kidney

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The pathogenesis of diabetic nephropathy is still incompletely understood and much of the experimental insight has been obtained from animal models. Clinically, diabetic nephropathy is characterised by persistent proteinuria, hypertension and finally progressive renal function impairment. The initial change in renal hemodynamics observed in diabetic nephropathy is glomerular hyperfiltration, i.e. an increase in glomerular filtration rate. Alpha-lipoic acid (LA) is a naturally occurring and unique antioxidant because it has beneficial effects on fuel metabolism and renal function. The Goto–Kakizaki (GK) rat is a lean, hyperglycaemic, hyperinsulinaemic, normotensive experimental model of type 2 diabetes with robust functional and structural renal changes. This study was conducted to investigate the effects of LA on renal function in diabetic and hypercholesterolemic GK rats. Carbohydrate and lipid metabolism, urinary albumin excretion, kidney weight, and creatinine clearance were assessed as well as plasma and urinary parameters of oxidative stress in non-diabetic controls (Wistar rats), untreated GK diabetic and atherogenic diet (AD) fed GK rats (AD only, alpha-lipoic acid and vehicle). Urinary albumin excretion was significantly higher in GK rats than in Wistar rats, aggravated with atherogenic diet and reverted with LA. Oxidative stress levels were increased in GK and GK hypercholesterolemic groups and also reverted with the antioxidant. Creatinine clearance was significantly lower in GK rats treated with AD than in Wistar rats at this age, while no further differences where observed. Kidney weight and fasting blood glucose levels were higher in diabetic and hypercholesterolemic rats, and reverted with LA. We conclude that alpha-lipoic acid improves albuminuria by reducing oxidative stress.

UDCA restores hepatic and mitochondrial GSH depletion through γ-GCS up-regulation in BDL

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Introduction. Cholestasis results in intrahepatic accumulation of potentially toxic bile acids, inducing hepatocyte apoptosis, necrosis and biliary fibrosis. Hydrophobic bile acids stimulate the generation of peroxides in hepatocytes and in liver mitochondria (mit_{liver}). *Aims*. 1) to quantify GSH depletion and mitochondrial impairment in BDL; and 2) to determine the protective effect of UDCA against BDL. *Methods*. 3 groups of rats: SHAM, BDL and BDL+UDCA (2.5g/kg). The experimental period lasted 28 days. Rate of peroxide production was determined in mit_{liver}. Mitochondrial and liver homogenate GSH and GSSG were measured. Real-time PCR of γ-GCS and γ-cystathionase (γ-Cys) mRNAs was performed. Western blot was performed using an anti-γ-GCS. *Results*. The rate of peroxide production in mit_{liver} BDL was twice as SHAM and UDCA treatment prevented such increase. Hepatic GSH was significantly lower in BDL *vs* SHAM; UDCA partially reversed this effect. A mirrored pattern was observed for GSSG levels, partially reversed by UDCA. The same changes were observed in mit_{liver}. BDL led to decreased expression of γ-GCS and γ-Cys mRNAs. UDCA prevented the decrease in γ-GCS mRNA, but not that in γ-Cys mRNA. UDCA partially restored γ-GCS protein decrease. *Conclusions*. UDCA treatment up-regulates γ-GCS expression and increases GSH synthesis in SBC. UDCA treatment prevented the increased mit_{liver} peroxide production observed in BDL.

Non-alcoholic steatohepatitis (NASH) induces mitochondrial oxidative stress, phospholipid alteration, and respiratory chain dysfunction

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Non Alcoholic Steatohepatitis (NASH) is recognized as a cause of chriptogenetic cirrhosis. It has been suggested that mitochondria, being the main source of ROS released in NASH and playing a key role in free fatty acid oxidation could participate to liver cell damage. We evaluated mitochondria respiratory chain activity and phospholipid change in mitochondria membrane in a diet model of NASH. Male Wistar rats assumed high-fat methionine and cholin deficient diet for 4-8 weeks. NASH was confirmed by AST and ALT measurement and histology. Mitochondria respiration was measured by Clark's electrode; phospholipids content and Reduced (GSH) and Oxidized (GSSG) glutathione level was also measured. Respiratory Control Index (ICR) was significantly higher in NASH than in control rats as well as complex II and IV activity. Mitochondria GSH and GSSG level were respectively higher and lower as compared to control rats. Analysis of phospholipid content of mitochondria membrane showed a significant reduction of phosphatidylcholin and phospha-tydilserine, and increase of Phosphatydilethanolamine and this condition could be responsible for mitochondria dysfunction. Moreover, change in lipid content and in respiratory activity related to increase of free radical production that produces GSH consumption. Our data demonstrates that NASH associates whit mitochondria lipid modification and respiratory chain alteration that induce oxidative stress, suggesting a key role of mitochondria in NASH pathogenesis.

Ischemic post-conditioning is effective in protecting the kidney against ischemia/reperfusion injury

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Improving the ability of the kidney to tolerate ischemic injury has important implications. We studied the effects of postconditioning on renal I/R and the role of mitochondria in the protection. Male wistar rats were assigned to I/R or post-conditioning (P-I/R), subjected to right nephrectomy and left renal pedicle complete occlusion by Schwartz's clip. In P-I/R group, postconditioning was performed treating the rats by 3, 6 and 12 min of reperfusion consecutively, separated by 5 min of reocclusion before definitive reperfusion. Rats were sacrificed at the end of ischemia and 24, 48 and 96 hours later. Creatinine and histology were performed as functional and morphological parameters respectively, and mitochondria respiration was measured by Clark's electrode. In the I/R group creatinine significantly increased during reperfusion as compared to protected rats. Morphological score showed a diffuse necrosis in non protected rats whereas a significant reduction of the necrosis occurred in P-I/R group. Respiratory Control Index and activity of Complex I, II and IV were measured; the analysis revealed that postconditioning preserves the mitochondria respiratory chain function, particularly restoring the complex II and IV activity which is significantly reduced in non protected group. Our results show that postconditioning protects, in vivo, the kidney against I/R injury by preventing mitochondrial dysfunction. Accordingly, we suggest that postconditioning could attenuate the renal damage during I/R procedures.

Quinone reactions with respiratory tract lining fluid antioxidants

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Quinones associated with airborne particulate matter (PM) have been proposed to contribute toward their observed pulmonary toxicity. To examine their potential to cause oxidation reactions at the air-lung interface we investigated their capacity to deplete ascorbate (Asc) from a series of models reflecting the antioxidant network within human respiratory tract lining fluids. A panel of quinones [9,10-phenanthroquinone (9,10-PQ), 1,2-naphthoquinone (1,2-NQ), 1,4-naphthoquinone (1,4-NQ) 1,4-benzoquinone (1,4-BQ) and 1,4-chrysenequinone (1,4-CQ)] were incubated with 200mM Asc, or composite antioxidant solutions containing Asc plus urate (UA), Asc plus glutathione (GSH), or all three antioxidants (AA+UA+GSH), all at 200mM, pH7. The loss of Asc with time was followed by monitoring the absorbance at A265nm, for 2h (37°C), at a range of quinone concentrations: 0.01-20mM. All quinones significantly depleted Asc from each of the antioxidant models with the following reactivity hierarchy: 1,2NQ > 1,4BQ > 9,10PQ > 1,4NQ > 1,4CQ. In the presence of GSH the rate of Asc depletion by 1,4-BQ, 1,4-NQ and 1,2-NQ was significantly reduced ($P<0.05$), whilst there was no impact of co-incubation of GSH on the rate of Asc oxidation by 9,10-PQ or 1,4-CQ. This study demonstrates the relative potential of particle-associated quinones to cause potentially damaging oxidations at the air-lung interface. It also highlights the protective effect GSH may play against certain electrophilic quinones.

Effects of dihydroquercetin on oxidation-induced changes of human red blood cells

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Flavonoid dihydroquercetin (DHQ) is a well-known scavenger of oxygen free radicals. In this study, the influence of DHQ (25 and 50 microM) on oxidative damage of human red blood cells (RBCs) in the presence of phenazine methosulfate (PMS) (25-100 microM) and ascorbic acid (10 mM) was investigated. The following parameters were assessed: accumulation of methemoglobin (MetHb) and 2-thiobarbituric acid reactive substances (TBARS); density/volume distribution of RBCs by phtalate method of Danon and Marikovsky (i), fluorescent-activated cell sorter (FACS) analysis (ii) and osmotic lysis curves migration assay (iii). Incubation of RBCs in the presence of PMS and ascorbic acid in Ca^{2+}-enriched medium resulted in a significant dehydratation of cells, decrease in forward light-scattering in FACS and shift of osmotic lysis curves towards progressively lower tonicity. These effects were abolished in the presence of clotrimazole (10 microM), a specific Ca^{++}-dependent K^{+} (Gardos) channel inhibitor. DHQ demonstrated the ability to protect RBCs against oxidation-induced volume changes (5-20%) and membrane peroxidation by lowering the accumulation of TBARS (about 20%). In the same time it had little influence on the accumulation of MetHb. Thus, DHQ owing to its antioxidant properties can prevent in vitro RBCs damage by oxygen free radicals.

Reactive oxygen species scavenging properties of newly synthesised nitrogen compounds

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Several pathologies result from an excessive intracellular production of reactive oxygen species (ROS). So, the search for new substances that can act against them, beyond the cells natural defences,has been increasing in the last few years. In this study we used new nitrogen compounds, from organic synthesis, to evaluate their real capacity as ROS scavengers. The new compounds were screened for their citotoxicity using LDH release test, in L929 cell line. The non-toxic ones were subsequently evaluated for their antiradical activity, determined by the DPPH discoloration method. Some of them presented an antiradical activity far superior to the traditional antioxidants α-tocopherol and trolox. At the IC50 concentration previously determined by the DPPH reduction test, the compounds revealed also as very effective in scavenging hydroxyl radicals, assessed by the 2-deoxy-D-ribose degradation assay. We selected the compounds with better overall performance and used them to monitor their protective effect on intracellular ROS formation in a PC12 cell model. The results obtained by flow cytometry, using the fluorescent probe H2DCF-DA, show that the compounds succeeded in preventing intracellular ROS formation induced by the oxidant pair ascorbate/iron. Altogether, these data are indicative of a quite good antioxidant potential for the new compounds that encourages complementar investigation.

JPS and FMA are supported by FCT grants SFRH/BD/17174/2004 and SFRH/BD/3185/2000, respectively.

Spectrophotometric assay to probe the redox status of cells *in vitro*

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Copper macrocycles were investigated as potential *in vitro* sensors of oxidative stress. The water-soluble and -stable copper (II) complex selected has square planar geometry and a redox potential coinciding with that predicted for physiological oxidants and antioxidants (~0.9 V). Reduction to the tetrahedral copper (I) form by ascorbic acid (100 – 500 μM) and GSH (150 – 750 μM) in water, phosphate buffer, phosphate buffered saline and phenol red-free RPMI medium was monitored spectrophotometrically. This was seen as loss of the major peak at 415 nm and also a lesser peak at 600 nm, so a green to colourless transition. Reoxidation in air was observed and in water 38% recovery of the 415 nm peak achieved using 650 μM HOCl.

The toxicity of the copper (II) compound was tested on U937 and THP1 cells, using the MTT and an ATP based assay. After four hours with 2.0 mM compound the cells were ~25% viable judged by the MTT assay, but the more sensitive ATP method showed almost complete depletion of this metabolite above 0.25 mM. This discrepancy will be investigated further, but work is already ongoing to use the compound to inform on the redox status of cells exposed to different conditions. Synthesis of an equivalent water-soluble and -stable copper (I) complex is also planned to allow more quantitative data to be gained for cellular oxidants.

Aerobic reactions of MnSOD with NO: peroxynitrite formation, enzyme inactivation, and tyrosine residue modifications

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Manganese superoxide dismutase (MnSOD) is the SOD isoform found in mitochondrial matrix of eukaryotes and in a variety of prokaryotes. The biological role of SOD is detoxification of superoxide radical ($O_2^{\cdot -}$) by converting it into H_2O_2 and O_2. In various diseases for which oxidative stress and overproduction of NO is characteristic, MnSOD was reported to be tyrosine nitrated, aggregated and inactivated, which further amplify the consequences of oxidative stress. In a previous study we have demonstrated that MnSOD (*E. Coli*) exposed to NO under the anaerobic conditions assists the conversion (disproportionation) of $NO^{\cdot}$ into NO^+ and NO^- species (Niketic et al., Free Rad. Biol. Med. 27 (1999) 992-996). In the present work we show that upon aerobic MnSOD exposure to NO peroxynitrite and H_2O_2 are generated in the nitroxyl-dependent fashion. The peroxynitrite generated in the vicinity of enzyme active site, causes nitration and oxidation of enzyme tyrosine resi-dues resulting in enzyme inactivation and aggregation. Collectively, these findings suggest that interactions of MnSOD with $NO^{\cdot}$ may represent a part of the protective mechanisms against high cellular $NO^{\cdot}$ levels. Furthermore, they provide com-pelling argument supporting the direct involvement of $NO^{\cdot}$ in the generation of nitrating species in MnSOD exposed to $NO^{\cdot}$.

Sodium-dependent vitamin C transporters Specific functions for the two isoforms in skin

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Vitamin C is an effective antioxidant and an essential cofactor in numerous enzymatic reactions. It comprises in two major forms: L-ascorbic acid, the reduced form, and L-dehydroascorbic acid, the oxidized form. Humans and other primates have lost the ability to synthesize vitamin C as a result of a mutation in the gene encoding for L-gulono-γ-lactone oxidase. In humans, two sodium-dependent vitamin C transporters (SVCT1 and SVCT2) were identified recently, but their roles in skin have as yet not been elucidated. Here we analyze the expression of SVCTs in skin biopsies using real-time PCR, Western Blot and immunofluorescence. Furthermore, primary human cells were used to assess their L-ascorbic acid uptake kinetics. SVCT1 was primarily found in the epidermis expressed by basal and suprabasal keratinocytes, whereas SVCT2 expression could be detected in the epidermis expressed by keratinocytes and in dermis expressed by fibroblasts and endothelial cells. Up-take experiments with 14C-labelled L-ascorbic acid revealed a Km of 75μM and a maximal velocity (Vmax) of 36nmol/min/ well for SVCT1 in primary human cutaneous cells. L-ascorbic acid affinity of SVCT2 was higher compared to SVCT1 (K_M = 44 μM) and V_{max} lower as for SVCT1 (V_{max} = 4nmol/min/well). In keratinocytes, SVCT1 was found to be responsible for vitamin C transport activity, although SVCT2 gene expression was higher. In summary, our data indicate that the two vitamin C transporter isoforms fulfill specific functions in skin: SVCT1 is responsible for epidermal vitamin C supply, whereas SVCT2 mainly facilitates vitamin C transport in the dermal compartment.

Evaluation and clinical study on antioxidant of the Artemisia Absinthium extract

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The plant of Artmesia absintium L is widely employed in Armenia popular medicine as a simulator, a tonic and remedy for digestion debility. The aim of presented work is to investigate the antioxidant effect of defenses of the lipid contained biological target of the water extract of Artemisia Absinthium (EAA). There were used the following experimental approaches: 1. The spectrometric method of determination of both products of lipid peroxidation (LPO)- diene conjugate (DC) and malone dialdehyde (MDA). 2. The chemiluminescence methods analysis of inhibition LPO by extracts. 3. The method of measure of stability of erythrocyte membranes from human blood. During influence of EAA with ended concentration of 10 mg/mL was noticed it suppressed activity by decreasing of the both concentration of DC on 38% and of MDA on 46%. Then was investigated the antioxidant activity of EAA in depend on its termal processing. The cold EAA showed 33% inhibition of generation of oxygen free radicals of cow brain homogenate. In case of termal at 100C for 15 min the EAA showed 50% of inhibition. The approximation of kinetic curved to the exponent with polynomial degree looks was allowed to evaluate the relative length of kinetic chain of oxidation, which increased is depend on duration the time of termal processing. The termal EAA increasing in the degree of browning and did not produce chemiluminescence. The cold (EAA) have very high level of chemiluminescence – 100-120 impulse/sec. The kinetics if inhibition of oxygen-dependent processes in erythrocyte suspensions will be investigated by using H_2O_2 initiators. The stability of erythrocytes membranes was arrived in case of termal EAA. There results can be useful in evaluation of activities drug plant in antioxidant therapy.

Stobadine prevents copper-induced oxidative damage on liver cells and tissue

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Pyridoindole derivative stobadine possesses antioxidant and radical-scavenging activity. In this work we investigated the efficiency of stobadine to prevent lipid peroxidation and liver cells and tissue damage. Oxidative stress is often considered as a pathophysiological mechanism of liver damage; while during enzymatic degradation of different drugs and toxic compounds free radicals can be created, thus damaging cells and liver function. Primary culture of liver cells was established for this purpose. Electron microscopy showed that the cell population varied in size and ultrastructural features, but almost all cells showed signs of hepatocyte differentiation. Intensity of lipid peroxidation caused by copper was determined by measuring malondialdehyde (MDA) with HPLC method. Cell viability was in parallel examined by Trypan blue staining. Efficiency of stobadine was compared with known antioxidant vitamin E. In our experimental conditions, stobadine acted as an antioxidant decreasing lipid peroxidation in cells and liver tissue, working when added before and after beginning of oxidation. Vitamin E was less efficient then stobadine and decreased lipid peroxidation only when given before copper and only in cell cultures, while in tissue homogenates it did not acted protectively at all. With this study we have shown that stobadine had a considerable protective effect against lipid peroxidation caused by copper. Such characteristic makes stobadine a possible hepatoprotective compound, which should be further evaluated in vivo.

Antioxidant properties of bioactive 1,4-dihydroisonicotinic acid derivatives

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1,4-Dihydroisonicotinic acid derivatives (1,4-DHINA) – a novel class of bioactive nitrogen heterocycles are compounds closely related to derivatives of 1,4-dihydropyridine (1,4-DHP), which are well-known calcium channel antagonists. However, 1,4-DHINA are not calcium channel antagonists, they have very low toxicity and act as antimutagens, while their activity principles are not explained entirely. The present study was performed to obtain data on 1,4-DHINA's antioxidant activities and to evaluate in vitro their bioactivity through possible influence on reactive oxygen species. The following compounds were tested: 2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydroisonicotinic acid sodium salt (Ia); sodium 2-(2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine-4-carboxamido)glutarate (Ib) and sodium 2-(2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine-4-carboxamido)ethane-sulphate (Ic). Radical scavenging and antioxidative properties of 1,4-DHINA derivatives were evaluated by several assays: N,N-diphenyl-N'-picrylhydrazyl (DPPH.); deoxyribose degradation; ABTS radical-cation scavenging; ANTIOX-CAP; MTT for cell viability and malondialdehyde determination by HPLC method with fluorescence detection for copper-induced lipid peroxidation. The results showed that Ia was the most potent antioxidant, with activity comparable to Trolox. Because the effects of Ia were observed in the concentration range that did not affect the growth of HeLa cells, while it reduced copper induced lipid peroxidation, Ia appears to be perspective for further antioxidant activity studies in vivo.

Selective inhibition of TNF-α-induced endothelin 1 upregulation in rat brain capillary endothelial cells by *N*-acetylcysteine is associated with reduced phosphorylation of NFκB p65 at Ser276

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N-acetylcysteine (NAC) is neuroprotective in acute brain injury models such as experimental bacterial meningitis, despite its poor blood-brain barrier permeability. Cerebral blood flow reduction by endothelin 1 (ET-1) appears to be a major contributor to the neuronal injury in these models. Since ROS have been implicated in regulation of ET-1 gene expression, we were interested whether NAC inhibited TNF-α-induced ET-1 upregulation in rat brain capillary endothelial cells (rBCEC4), and what the potential signaling mechanisms were. Intriguingly, NAC selectively inhibited upregulation of ET-1 mRNA and protein, while upregulation of iNOS, another NFκB-regulated gene product, was unaffected. In line with these results, NAC neither affected degradation of IκB, nor nuclear translocation of p65. However, NAC inhibited NFκB DNA-binding activity. Inhibition of DNA-binding activity by phosphatase treatment of nuclear extracts suggested that phosphorylation of NFκB is required for ET-1 upregulation. Treatment of cells with H89 dose-dependently inhibited TNF-α-induced upregulation of ET-1 but not of iNOS, suggesting a mandatory role for MSK1 in ET-1 upregulation. Indeed, H89 and NAC inhibited phosphorylation of p65 at Ser276, which has recently been shown to be important for transcriptional activity of a specific subset of NFκB-dependent genes and a target of MSK1.

Redox regulation of Bcl-x_L expression in pulmonary artery smooth mucle

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Hypoxic pulmonary hypertension is characterized by thickened pulmonary arterial walls due to increased number of pulmonary artery smooth muscle cells (PASMC). Serotonin (5-HT) and endothelin-1 (ET-1) induce proliferation of PASMC via reactive oxygen species (ROS). Nitric oxide (NO), in contrast, suppresses growth and induces apoptosis. Mechanisms of apoptotic regulation in PASMC are not well understood. We examined the regulation of an anti-apoptotic protein Bcl-x_L. Treatment of cells with NO donors downregulated Bcl-x_L gene expression. The Bcl-x_L promoter contains GATA binding sites, and NO inhibited GATA-4, which is expressed in PASMC, but not in SMC from the systemic circulation. NO actions to influence the GATA activity involve the suppression of GATA-4 gene transcription, as NO downregulated GATA-4 mRNA expression and GATA-4 promoter-dependent luciferase activity. Neither NO-induced downregulation of GATA nor Bcl-x_L was inhibited by KT5823, suggesting that protein kinase G-independent pathways are involved. Adenovirus-mediated overexpression of GATA-4 attenuated the NO-suppression of Bcl-x_L expression, providing direct evidence for the role of GATA-4 in Bcl-x_L gene transcription. 5-HT and ET-1 activated GATA-4 via ROS, enhanced Bcl-x_L expression, and protected PASMC against apoptosis. Bcl-x_L expression was also enhanced in remodeled pulmonary arteries from rats exposed to chronic hypoxia. These results suggest that inducers of pulmonary hypertension enhance Bcl-x_L transcription via activating the ROS-GATA-4 pathway, while NO inhibits Bcl-x_L expression by suppressing GATA-4 gene transcription.

The zinc, copper, and selenium concentrations in patients after gastrointestinal tract surgery

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The trace elements like zinc, copper and selenium are the cofactors of the main antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx). The surgery can lead to disturbances in these elements homeostasis. Based on this the aim of study was to determine the influence of operation on these trace elements. The material was obtained of 64 operated patients because of gastrointestinal tract lesions in the Surgery Department of Jan Bo¿y Hospital in Lublin. The blood was collected in the first day before operation and the first, the third, the fifth and the seventh day after surgery. Zinc, copper and selenium concentrations were measured by atomic absorption spectrophotometry (AAS). It has been show that surgical operation decreased trace element concentrations in comparison to them concentrations measured before an operation. The lowest concentration of zinc, copper and selenium were observed in the first, third, fifth day after surgery, respectively. These changes were statistically significant.

Antioxidant enzyme activities in patients after gastrointestinal tract surgery

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The surgery influences on generation of reactive oxygen species (ROS) and on the antioxidant defence system efficiency. The intensification of ROS production in the course of operation can lead to decrease of antioxidant enzyme activities. Based on this the aim of study was to determine the influence of operation on antioxidant enzyme activities. The material was obtained of 64 operated patients because of gastrointestinal tract lesions in the Surgery Department of Jan Bo¿y Hospital in Lublin. The blood was collected in the first day before operation and the first, the third, the fifth and the seventh day after surgery. Antioxidant enzyme activities like superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured spectrophotometrically using Randox Laboratories Ltd. kits. The surgical operation resulted in decrease of activities of tested antioxidant enzymes in comparison to them activities measured before surgery. The lowest SOD and GPx activities were observed in the third and fifth day after operation, respectively. These changes were statistically significant.

Organophosphate induces oxidative stress in exposed agriculture workers

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Organophosphates (OPs) are irreversible inhibitors of erythrocyte cholinesterase and plasma butyrilcholinesterase (BuChE), thus they are generally considered as the most toxic of all pesticides. Although acute OP toxicity is primarily due to AChE inhibition in the nervous system, the induction of oxidative stress may also be a potential mechanism of OPs deleterious toxic effects. The aim of our study was to investigate the oxidant stress (serum thiobarbituric acid reactive substances (TBARS) levels) and antioxidant status both in a group of viniculture and tobacco workers (n = 65) who are occupationally exposed (duration of exposure = 4-36 years, dimension of the OP applied area = 3-700 acre of land) to OPs and in healthy age matched controls (n = 30). While BuChE activity was significantly ($p < 0.013$) reduced in the OP exposed group as an indicator of exposure, TBARS levels were significantly higher ($p < 0.037$) than the healthy controls. There was no significant difference in TAO activity between the groups. The dimensions of the OP applied area and duration of exposure did not seem to have a significant effect on any of the oxidative stress parameters. It should be noted that although pests are the target for OP usage, humans are the non–target potentially effected group that may be exposed seriously such as the case of agricultural practice in workers. This potential oxidative inducing effect should be carefully monitored in OP exposed workers and preventive strategies to maintain the oxidant-antioxidant balance such as supplementary antioxidant agents may be considered in occupational medicine applications.

This study was supported by Government Planning Organization of Turkey (03/DPT).

Nitric oxide derived from endothelial nitric oxide synthase initiates anti-inflammatory effects of ethanol preconditioning

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We have shown that ethanol preconditioning (EPC) prior to ischemia/ reperfusion (I/R) prevents leukocyte-endothelial cell adhesive interactions (LEI) in the small intestine. The aim of this study was to determine the role of eNOS-derived NO as an initiator of the anti- inflammatory effects of EPC in I/R. Ethanol was administered to wild- type (WT) or eNOS-/- mice. 24 hrs later, I/R was induced by clamping the superior mesenteric artery for 45 min followed by reperfusion. The numbers of fluorescently-labeled rolling and adherent leukocytes were quantified in single postcapillary venules of the small intestine. In separate studies in WT mice, a NOS inhibitor (L-NIO) was administered before ethanol administration. SNAP (a NO donor) was administered in lieu of ethanol gavage. I/R was then induced on Day 2 in all experiments. As a result, I/R induced marked increases in LEI as compared with sham in WT mice. The increased LEI was completely prevented by EPC. The anti-inflammatory effects of EPC were abolished by coincident treatment with L-NIO during EPC. In eNOS-/- mice, I/R also elicited an increase in LEI in comparison to sham. Although SNAP mimicked the protective effects of EPC on postischemic LEI in both WT and eNOS-/- mice, EPC failed to prevent postischemic LEI in eNOS-/- mice. These results indicate that the anti-inflammatory effects of EPC exhibited during I/R on Day 2 are initiated by eNOS-derived NO that is formed during the period of ethanol exposure on Day 1.

Protective effects of sulforaphane against oxidative stress-induced apoptosis in human neuronal cells

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Oxidative stress due to generation of reactive oxygen species is implicated in neuronal apoptosis and progression of neurodegenerative disorders such us Alzheimer's and Parkinson's diseases. Recent studies have demonstrated that dietary compounds with antioxidant actions may represent treatment avenues for neurodegenerative diseases. In this study, we evaluated the protective effects of Sulforaphane (SF), a isothiocyanate found in cruciferous vegetables, against H_2O_2-induced apoptosis, in terms of loss of mitochondrial membrane potential ($\Delta\psi m$), activation of caspase-9, -3 and DNA fragmentation, in a human neuroblastoma cell line (SH-SY5Y). Moreover, to evaluated the indirect antioxidant activity of SF, we determined the Total Antioxidant Activity (TAA) of cytosolic fraction and levels of glutathione (GSH) of neurons. Treatment of SH-SY5Y cells with SF (0,6-5 μM) for 24 h before treatment with H_2O_2 (300 μM) showed a dose-dependent inhibitory effect on H_2O_2-induced $\Delta\psi m$ loss, caspase-9 and -3 activities. The results also demonstrated that similar concentration of SF inhibited DNA fragmentation induced by H_2O_2. Furthermore, SF induced an increase of TAA and levels of GSH, in a dose-dependent manner, with a maximun increase of 2- and 3-fold, respectively. These results highlight that SF has strong in vitro neuroprotective effect against oxidative cell damage.

Supported by Fondazione del Monte di Bologna e Ravenna (Italy)

COX-2 Inhibitory effect on human hepatocellular carcinoma cell growth can be mediated by the $p27^{Kip1}$ overexpression

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Treatment of cancer is limited by either intrinsic or acquired expression of multiple drug resistance transporters. One of the most studied mechanisms concerns the overexpression of a membrane-associated glycoprotein, P-glycoprotein (P-gp), known as the multiple drug resistance (MDR1) phenotype. The induction of MDR phenotype was found to be associated with the appearance of the angiogenetic phenotype in human hepatocellular cell (HCC) lines, as shown by the increased expression of COX-2 and iNOS, suggesting susceptibility to COX-2 inhibitors (Fantappiè et al., Hepatology 2002, 35, 843-852). Ciclin dependant kinase inhibitors, tumour suppressor genes regulating tumour growth, angiogenesis and metastasis, have been proposed to be predictors of patient outcome. Because $p27^{Kip1}$, a CDKI inhibitor of Cip/Kip family, is frequently inactivated in HCC and it is involved in the angiogenetic process, here we investigated the $p27^{Kip1}$ expression, by immunohistochemical analysis, in parental drug-sensitive (P5) HCC cell lines and in P5-derived MDR cells P1 (cells maintained in the presence of 0.5 μg/ml of doxorubicine) after exposure of cells to the specific COX-2 inhibitor, celecoxib, in the presence or in the absence of fetal bovine serum. We found that exposure to celecoxib caused nuclear $p27^{Kip1}$ overexpression and a significant inhibition of cell growth in MDR positive cells. Similar results were also observed in experiments performed in the absence of fetal serum. These results suggest that nuclear $p27^{Kip1}$ is involved in the regulation of cell replication in human derived HCC with the expression of MDR phenotype and that the anti cancer effect of COX-2 inhibitors, such as celecoxib, could be mediated by overexpression of $p27^{Kip1}$. If it were confirmed, $p27^{Kip1}$ could be one of the possible targets for gene therapy in HCC.

Antioxidants in the protection from diabetic nephropathy

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Glycoxidation is believed a key factor in long-term diabetic complications. We previously demonstrated the protective effect of some antioxidants (oxerutin, N-acetylcysteine, and N-acetylcysteine+taurine) on renal glycoxidative damage, evaluated by immuno-histochemistry (carboxymethyllysine, HNE- and MDA-adducts) and morphometry (glomerular enlargement, increased apoptotic rate and decreased cell density). This prompted us to investigate the possible mechanisms of protection in primary human mesangial cell cultures. These cells have been exposed to diabetes-like conditions: Ribated BSA and High Glucose. Markers of glycoxidative stress and fibrogenic response are currently evaluated and correlated with cell viability and proliferation. Our preliminary results showed that: a) Both High Glucose and Ribated BSA induced the expression of Heme Oxygenase-1; b) Both High Glucose and Ribated BSA caused superoxide generation; c) Cell proliferation appeared slightly enhanced by both Ribated BSA and High Glucose; d) Viability appeared impaired neither in High Glucose nor Ribated BSA; e) Increased production of TGFbeta1 mRNA appeared both in High Glucose and in Ribated BSA. These preliminary results seems to indicate the generation of mild oxidative stress by diabetes-like conditions, with fibrogenic-oriented gene expression cell response.

Grants from University of Genova, Cofin 2003 (#2003067599_003 Coord. Scient. Naz. Prof. E. Bergamini), and FIRB 2001 (#RBAU01J BH8 _003 Coord. Scient. Naz. Prof. G. Poli)

Functional activity of mitochondrial nitric oxide synthase

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Nitric oxide added exogenously or produced by mitochondrial nitric oxide synthase (mtNOS) regulates mitochondrial electron transfer. The ability of mtNOS activity to modulate O_2 uptake and H_2O_2 production has been named "*functional activity of mtNOS*". Respiratory rates and H_2O_2 release were determined at maximal and minimal NO levels. Supplementation of state 3 mitochondria with L-arg and SOD decreased the respiration rate by 2-16%, whereas the addition of a NOS inhibitor and HbO_2, increased the O_2 consumption by 8-55% in heart, liver, kidney and brain mitochondria. These effects are explained by the inhibition of cytochrome oxidase by NO in a process that is competitive with O_2 (Antunes et al., PNAS 48:16774-9, 2004). The difference between the state 3 O_2 uptake with L-arg and SOD and with a NOS inhibitor and HbO_2 indicates the *mtNOS functional activity in the inhibition of cytochrome oxidase activity* (13-70%). Addition of L-arg increased state 4 H_2O_2 production by 9-28% in heart, liver, kidney and brain mitochondria, whereas the supplementation with a NOS inhibitor decreased H_2O_2 generation by 3-55%. The difference in H_2O_2 production between the conditions of maximal and minimal NO levels is termed *functional activity of mtNOS on the regulation H_2O_2 production* (18-68%). The effects on H_2O_2 production are explained by the NO inhibition of ubiquinol-succinate-cytochrome c reductase activity that enhances H_2O_2 production. The regulatory role of NO on mitochondrial functions extends from physiological to pathological situations. Simple respiratory determinations such as O_2 consumption and H_2O_2 production can be effectively used to assay effects of physiological or pharmacological treatments on mtNOS activity and mitochondrial function.

Mitochondrial metabolic states and membrane potential modulate mitochondrial nitric oxide synthase activity

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Mitochondrial nitric oxide synthase (mtNOS) catalyses nitric oxide (NO) production in mitochondria. Coupled respiring mitochondria release NO to the reaction medium; however, the relationship between the rates of NO release and mitochondrial energetics are not clear. The rates of NO production and release and their relationship with mitochondrial membrane potential were determined. The mitochondrial metabolic state regulates the rate of NO release from coupled mitochondria: NO release by heart, liver and kidney mitochondria was about 50% lower in state 3 than in state 4. The ratio of membrane potentials at different pH values significantly correlated with respiratory control and NO release ratios. The decrease in NO release in the state 4-state 3 transition was opposite to what is expected from the matrix acidification of the state 4-state 3 transition and the pH dependence of mtNOS activity: liver and kidney mtNOS show a 57% and 17% higher activity at pH 7.5 (state 3) than at pH 7.8 (state 4). Nitric oxide release and Rh-123 fluorescence were determined in the same experimental conditions: agents that decrease or abolish membrane potential (antimycin or CCCP) minimized NO release (0.48 nmol NO/min. mg protein), while the addition of oligomycin that produces mitochondrial hyperpolarization generated a maximal NO release (1.4 nmol NO/min·mg protein). Nitric oxide release was exponentially associated to membrane potential, and this regulation was marked at the physiological range of membrane potentials. In summary, the evidence reported here sustains the notion of a mitochondria-specific NOS (mtNOS) associated to the inner membrane and regulated by membrane potential. Correspondence to lbvaldez@ffyb.uba.ar

β-Amyloid peptide increases free radicals producing cell death by necrosis and apoptosis in primary culture of neurons and astrocytes

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The presence of senile plaques, consisted of β-amyloid peptide (Aβ) in different brain regions, is one of the principal characteristics in Alzheimer's disease (AD). The neurotoxin Aβ (Aβ-1.42) produces dendritic injury, synaptic lose and neural death. Actually the toxic mechanism of Aβ in brain continues not well understood. Using Aβ-1.42 and Aβ-40.1 as a control peptide, we demonstrate the intracellular toxic effect of Aβ in neurons and astrocytes in primary culture. Cells incubated with both peptides were marked with the following fluoresce products: mitotracker, to see mitochondrial aggregation, lysotracker, to stain lysosomes, propidium Iodine, to show necrotic cell death, anexin V, to note apoptotic cell death and hoechst 33342, for nucleus staining and confocal microscopy was used. In neurons in primary culture, we demonstrate here a mitochondrial aggregation with increase in apoptotic and necrotic cell death compared with control cells. Furthermore, a less lysotracker staining in treated cells was noted compared with not treated cells. Our results demonstrate that after lactate dehydrogenase (LDH) determination and MTT assay Aβ cells present a significant increase of cell death compared with control cells. Also treated neurons have an increase in peroxide production compared with control cells. Our results show that the toxic effect of the Aβ peptide is similar in neurons and in astrocytes, producing mitochondrial aggregation, a lower fluorescence intensity of lysosomes with an increase in apoptotic and necrotic cell death. The data will explain the deleterious effects of this toxic in all cell types in brain of Alzheimer's disease.

4 -Coumaric acid and Verapamil protect nuclear DNA and mitochondrial DNA from oxidative damage in human hepatocellular carcinoma cell lines

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Multiple drug resistant (MDR) phenotype expression and P-glycoprotein (P-gp) over-expression in the human hepatocellular carcinoma (HCC) cell clone P1(0.5), derived from the PLC/PRF/5 cell line (P5), are associated with a surprising significant ($p < 0.05$) mitochondrial DNA higher susceptibility to oxidative stress than the parental cell line. In fact, cell clone P1(0.5) shows an higher increase in intracellular vitamin E content than the parental cell line (Mazzanti R., et al., 1995). At the same time, P1(0.5) cells mitochondrial DNA higher susceptibility to the oxidative damage could be a phenomenon involved in the most aggressive human tumors typical genetic instability. This study evaluates the 4-Coumaric acid (a natural polyphenol) and Verapamil role in conferring resistance to oxidative stress in P1(0.5) and in P5 cells. Susceptibility to lipid peroxidation and oxidative nuclear and mitochondrial DNA damage were assessed by measuring the thiobarbituric-reactive substances (TBARS) concentration and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels at basal and after experimental conditions. Exposure to ADP-Fe^{+3} or ASC-Fe^{+2} increased TBARS and 8-OHdG content in the P5 cells and the exposure to ADP-Fe^{+3} increased 8-OHdG content in the P1(0.5) cells but, in any case 4-Coumaric acid (5 mM for 24 h), Verapamil (5mM for 24 h) for 24 h) medium enrichment abolished these effects. Our data show the role of these molecules in conferring protection from lipid peroxidation and oxidative nuclear and mitochondrial DNA damage on the human HCC cell line and suggest a their possible tumors prevention or treatment use.

Oxidant properties of metallic complexes of meso-tetrasulfonated porphyrins. Generation of reactive oxygen species ($HO^{\cdot}$, $^1O_2$)

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The objective of this study was to investigate the ability of Cu(II), Zn(II), Pd(II), Mn(II), Fe(II), Ni(II) and Co(II) complexes of meso-tetraphenylsulfonated porphyrins to generate the reactive oxygen species ($^{\cdot}OH$, $^1O_2$) under irradiation. The ability of these compounds to generated .OH in cell-free systems using luminol-chemiluminescence, were investigated. The irradiation of these compounds with visible light (two fluorescent lamps, model RPR-100) in presence of NADH (equimolar $1.0x10^{-4}$ M) produced hydrogen peroxide that could be detected by luminol-enhanced chemiluminescence in a Luminoskan Ascent luminometer (96-well plate, ThermoLabsystems Microtiter, Finland). These compounds are capable of producing singlet oxygen by energy transfer when they are irradiated with UV-A and visible light in the presence of molecular oxygen. This fact can be confirmed by trapping with histidine. We conclude that an oxidation of histidine is produced through photoexcitation of the metallized-sulfonated porphyrins acting as a singlet oxygen sensitizer (type II mechanism). This particular reaction with histidine can be regarded as a model for damage to cellular protein inflicted by photoexcited-derived porphyrin via formation of singlet oxygen. We compare this capacity with the reported data for the same reaction using Rose Bengal. This value can be used as a standard to determine the quantum yield singlet oxygen for all the complexes studied. The histidine model should be regarded simply as a test for oxygen dependent photosensitized damage to cellular protein.

Oxidized phospholipids in apoptotic cell plasma membrane trigger the formation of anti-phospholipid

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Circulating anti-phospholipid antibodies (aPL) are present in about 80% of patients with advanced alcoholic liver disease (ALD). However, they origins and clinical significance are still poorly understood. The observations that defects in the disposal of apoptotic corpses leads to the development of aPL prompted us to investigate whether ALD-associated aPL might recognize antigens in apoptotic cells. To this aim apoptosis was induced in HuT-78 human T-lymphoma and HepG2 hepatoma cells by, respectively, FAS ligation with CH11 monoclonal antibodies or the incubation with ethanol (400 mmol/L). Flow cytometry reveals that IgG from ALD patients with high aPL titers selectively bind to the surface of apoptotic, but not to viable cells. No binding is instead evident using either control or aPL-negative ALD sera. ELISA assays using as antigens different oxidized phospholipids show that ALD sera display high IgG reactivity against both oxidized cardiolipin and phosphatidylserine and to less extend against oxidized ethanolamine, while no differences in the recognition of oxidized phosphatidylcholine and oxidized inositol were appreciable between ALD and control sera. The pre-adsorption of aPL-positive sera with oxidized phosphatidylserine, but not with oxidized cardiolipin, lowered by about 50% aPL binding to apoptotic HuT-78 cells. No effect was instead observed by pre-adsorption with oxidation-protected phospholipids or with human serum albumin adducted with different lipid peroxidation products. Altogether these results suggest alcohol-induced hepatocyte apoptosis as possible cause for the development of anti-phospholipid auto-reactivity in ALD.

Two distinct mitochondrial pathways are involved in oxidized LDL-induced apoptosis. Role of calcium

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Apoptosis of vascular cells contributes to the instability of atherosclerotic lesion, plaque rupture and subsequent thrombosis. Among the different proatherogenic agents present in the plaque and potentially involved in apoptosis and plaque rupture, oxidized LDL play a major involvement since they behave as cytotoxins or "trojan horses", which trigger biphasic proatherogenic signalings like migration and proliferation of SMC at low concentrations, and apoptosis of vascular cells at higher concentrations, (both events existing in early and advanced atherosclerotic lesions). Apoptosis induced by oxLDLs is mediated by various signalings including an up-regulation of Fas ligand, caspase activation, cytosolic calcium deregulation, and the activation of stress kinases such as Jun and SAPK. We aimed to clarify the relationship between the oxLDL-induced calcium signal and induction of apoptotic pathways. We report that the calcium rise elicited by oxLDL activated 2 distinct calcium-dependent mitochondrial apoptotic pathways in human microvascular endothelial cells. OxLDLs induced calpain activation, subsequent Bid cleavage and cytochrome C release, which were blocked by calpeptin. Cyclosporin-A inhibited cytochrome c release, possibly by inhibiting the opening of the mitochondrial permeability transition pore (mPTP). Cytochrome c release in turn induced caspase-3 activation. In addition, oxLDLs triggered release and nuclear translocation of mitochondrial apoptosis-inducing factor through a mechanism dependent on calcium but independent of calpains, mPTP, and caspases. In conclusion, OxLDL-induced apoptosis involves 2 distinct calcium-dependent pathways, the first mediated by calpain/mPTP/cytochrome c/caspase-3 and the second mediated by apoptosis-inducing factor, which is cyclosporin-insensitive and caspase-independent.

Iron homeostasis and methionine-centered redox cycle in aging of head and neck organs

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Tissue injury, expressed as damage to DNA, proteins and lipids, accumulates with aging. We investigated age-related changes in 4 rat head and neck organs – esophagus, larynx, sternohyoid muscle and tongue. Iron homeostasis (including levels of iron and ferritin), as well as levels of methionine-centered redox-related proteins [including the methionine sulfoxide reductase (Msr) family of enzymes, and levels of thioredoxin (Trx) and thioredoxin reductase (TrxR)], were studied. In both sternohyoid muscle and tongue, a considerable age-related decrease in ferritin concentration was observed, while total tissue iron levels remained unchanged. Ferritin level in the esophagus was higher in the old group while it has remained unchanged in the larynx. Trx level did not change with aging in the sternohyoid muscle and tongue, while a dramatic decrease was observed in the other 2 organs. Marked age-related changes in both levels of MsrA and MsrB (with a much higher decrease in MsrB level) were observed in all organs. The esophagus is clinically related to the largest range of age-associated diseases among the studied head and neck organs. The total reduction in its anti-oxidative capacity corresponds well with a possible role to age-related free radical damage. Additional studies are being conducted in order to further determine the role of free radical damage in aging of head and neck organs.

The aldehyde 4-hydroxynonenal interacts with TGF-β1 on inducing apoptosis in human colon adenocarcinoma cells

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4-Hydroxynonenal (HNE) is implicated in the modulation of the expression of several genes involved in cell proliferation. This aldehyde exerts its antiproliferative effects mainly through the activation of JNK cell transduction pathway. During tumor progression, studies on human colon adenocarcinoma show a low tissue content of HNE that is constantly related to a decrease of the antiproliferative cytokine, TGF-β1. By this way tumor cells display a resistance to the growth inhibitory effect of these two molecules, which could represent an obstacle for neoplastic progression. We studied the combined action of 10 ng/ml TGF-β1 and 1 μM HNE in inducing apoptosis in CaCo-2 human colon adenocarcinoma cells and we deepened the involvement of HNE-related cell signal, JNK, and the main TGF-β1 signal pathway, Smads. The simultaneous treatment with both TGF-β1 and HNE amplified the apoptosis induced by TGF-β1 alone. Furthermore, cell co-treatment led to a marked amplification of both signaling pathways, as a result of a synergistic effect between the two trigger molecules. The pre-treatment with a JNK inhibitor, SP600125, in cells challenged with both the molecules, not only decreased JNK activation, but also lowered Smad4 signal and, noteworthy, quenched apoptosis induced by TGF-β1 and HNE co-treatment. In tumor cells the small part of TGF-β1-signaling still working could be improved by HNE-related JNK, which sustains Smads signal to enhance TGF-β1-related apoptosis, thus counteracting tumor progression.

Irradiation-induced damage of GAPDH

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The accumulation of damaged proteins is a hallmark in several neurodegenerative diseases and aging. Environmental stress like IR and UV-irradiation can lead to oxidative modifications of amino acids and in consequence to an accumulation of non-functioning proteins. Therefore, we analyzed the impact of different sources of irradiation on proteins using GAPDH as model enzyme. We choose the fragmentation, aggregation as well as the formation of protein-bound carbonyls within the model protein as readout for the irradiation induced damage. Fragmentation and aggregation were detected by Coomassie blue staining after SDS-PAGE. Protein bound carbonyls were measured by ELISA and immunoblotting analysis. The irradiation of the model protein with different doses of UV-A-, UV-B-, IR- and gamma-irradiation lead to different modification patterns. UV-A and IR induces almost no carbonyl formation in contrast to UV-B and gamma irradiation. Gamma irradiation causes an unspecific fragmentation and aggregation of GAPDH while UV-A, UV-B and IR lead to more distinct irradiation products. Therefore, we concluded that various environmental stressors affect different markers of protein oxidation.

Cytotoxicity and cytoprotective activity in naphthalenediols depends on their tendency to form naphthoquinones

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We report on the cytotoxicity and the protection against oxidative stress for members of the naphthalenediol family and the known antioxidant epigallocatechin gallate (EGCG). Compounds include the 1,2-naphthalenediol (1,2-ND), 1,4-ND, 2,3-ND, 1,8-ND and 1,4-dipropyl-2,3-naphthalenediol (DPND). The cell line is an adherent clone of rat pheochromocytoma (PC12-AC). Oxidative stress was induced by the peroxyl radical generator AAPH. The relative order of cytotoxicity was 1,4-ND > 1,2-ND > DPND > 2,3-ND > 1,8-ND > EGCG, with EC50's of 15, 40, 160, >250, > 250, >> 250 μM, respectively. In spite of their high toxicity, both 1,4-ND and 1,2-ND showed narrow zones of protective behavior whereas DPND, 2,3-ND and 1,8-ND and especially EGCG showed an extended protective range. The total protection obtained for the combination of cells/oxidative stressor/protective compounds (PC12-AC/AAPH/naphthalenediols) was defined by an integrated measure, the Cytoprotective Area (CPA). We relate the observed cytotoxicity and CPA to the different electronic structures of the naphthalenediols, characterized by the first and second bond dissociation enthalpies and the pKa's for parent (diol) and semiquinone. Since the 2,3- and 1,8-naphthalenediols do not form quinones, their cytotoxicity is much lower than for the compounds which do. Thus selected members of the naphthalenediol family show promise as antioxidants.

Heart adaptation to environmental hypoxia. Up-regulation of mitochondrial nitric oxide synthase

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The present study determines the time course and effect of chronic exposition to environmental hypoxia on heart mitochondrial NOS (mtNOS). The changes are considered along with other markers of adaptation such as hematocrit, heart contractility parameters and mitochondrial enzymatic activities. a) Hypoxia in hypobaric chamber: Rats were housed in a hypobaric chamber (53.8 kPa) and sacrificed at 2, 4, 8, 12 and 18 mo of age. Hypoxic rats showed 20-60% increased left ventricle mtNOS activity as compared with their normoxic siblings. Left ventricle NADH-cytochrome c reductase and cytochrome oxidase activities were not affected by hypoxia. The increase in mtNOS activity of hypoxic rats was associated to a retardation of the decline in the mechanical activity of papillary muscle upon aging and an improved recovery after anoxia-reoxygenation. b) Hypoxia at high altitude: Rats were transported to Cerro de Pasco, Perú (4340 m, 61.3 kPa) and sacrificed at 7, 14, 21, 42 and 84 days. Animals responded with arrest of gain weight, right ventricle hypertrophy (230%) and increased hematocrit (40%). High altitude increased (80%) heart mtNOS activity, whereas it did not affect heart cytosolic eNOS activity. There was an increase of 60% in the heart mitochondrial NOS expression. A linear correlation ($r^2 = 0.88$) between hematocrit and mtNOS activity was observed. In conclusion, environmental hypoxia triggers an adaptive response that involves the up-regulation of heart mtNOS.

Are 5-pyrimidinols better antioxidants than phenols also in model membranes and cells?

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The study of antioxidant involvement in human health has become very significant since free radicals have been suggested to play a role in heart, lung and neurodegenerative diseases and to be involved in cancer and ageing. Phenols are widely distributed in nature and constitute a very important class of radical scavengers. Besides these natural antioxidants, there are efforts in order to synthesize novel compounds, that have to be air-stable and react with free radicals more efficiently than phenols. Recent data suggest that switching from a phenol to a pyrimidinol structure may be a solution to this problem (1,2). A series of substituted 5-pyrimidinols were synthesized, and data obtained in homogeneous phase showed that they act as effective chain-breakers with the desired features (2). As they likely have promising pharmacological activity (anti-inflammatory and citoprotective), we tried to verify their antioxidant activity in model membranes. In particular, we evaluated the inhibition of oxygen consumption during autoxidation of egg lecithin vesicles initiated by the thermolabile azocompound AAPH. All the tested compounds behaved as good chain-breaking antioxidants, with different ability depending on their different lipophilicity. Since MTT assay excluded cytotoxicity, the antioxidant activity of 5-pyrimidinols was proved in different cell types.

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Aspirin-like drugs inhibit lipolysis through reactive oxygen species generation

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Isolated rat adipocytes incubated with NSAID -aspirin, naproxen, nimesulide and piroxicam- at concentrations within their therapeutic range, and in a dose-response manner, inhibited epinephrine-stimulated and cAMP-activated glycerol release (Zentella de Pina et al., 2002). The purpose of this work was to obtain insight into the mechanism of action of NSAID on lipolysis. Catalase impaired the inhibitory effect of NSAID on DcAMP-activated lipolysis, and NSAID generated H_2O_2 in dose-response manner with a peak at 10-6 M. In plasma membrane from isolated adipocytes, NSAID stimulated in dose-dependent fashion an NADPH-dependent H_2O_2 generation system (NOX), cAMP partially impaired this stimulating effect. With cell free preparation from isolated adipocytes and in dose-dependent manner, H_2O_2 decreased cAMP-dependent PKA activation, and hence, PKA catalytic activity. Translocation of phosphorylated hormone-sensitive lipase from cytosol to storage-droplets was decreased by H_2O_2. In conclusion, through activation of NOX, four NSAIDs raised the generation of extracellular H_2O_2 under subtoxic conditions and reciprocal cross-talk in lipolysis regulation at the level of adipocyte: H_2O_2 blunt the down stream activation of cAMP to promote lipolysis, and cAMP restrain NOX system devoted to lessen lipolysis. A protagonic role of H_2O_2 regulating one of the most important intracellular signaling mechanism in ubiquitous cells is reported.

This work was supported partially by Grant IN-224903, DGAPA. UNAM. and 45003-M CONACYT México.

Progress on nitric oxide sensors and their biomedical applications

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Nitric oxide is a key intercellular messenger in the human and animal bodies. The identification of NO as the endothelium-derived relaxing factor (EDRF) has driven an enormous effort to further elucidate the chemistry, biology and therapeutic actions of this important molecule. It has found that nitric oxide is involved in many disease states such as such as chronic heart failure, stroke, impotent (erectile dysfunction). The bioactivity of nitric oxide intrinsically linked to its diffusion from its site production to the sites of action. In last two decades, NO has been the target of intensive research work aimed at monitoring its role in biological systems. Accurate reliable in real time detection of NO in various biological systems is therefore crucial to understanding its biological role. However, the instability of NO in aqueous solution and its high reactivity with other molecules can cause difficulties for its measurement depending on the detection method employed. Although a variety of methods have been described to measure NO in aqueous environments, it is now generally accepted that electrochemical detection using NO-specific electrodes is the most reliable and sensitive technique available for real-time in situ detection of NO. Since the first commercial NO electrode-based amperometric detection system was developed in 1992, many new electrochemical nitric sensors have been invented and commercialized. Here we describe some of the background, principles in electrocehmical NO sensors design, fabrication, methodology. Problem and progress on NO sensors are reviewed. Biomedical applications of NO sensors are given, including detection of NO in invo and in vitro such as measurement of NO in brain, human artery, human stomach, under human skin, kidney tubular, cultured cells.

Resveratrol analogs: Correlation between antioxidative and chemopreventive activities

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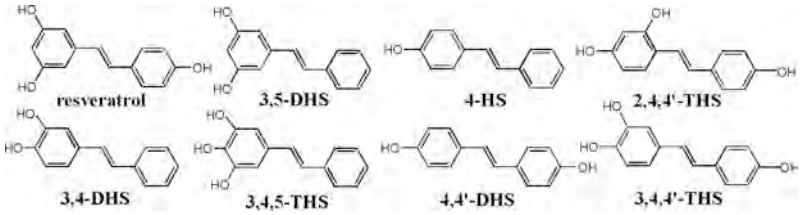
Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) is a key antioxidant in red wine which is believed to be responsible for the low incidence of cardiovascular diseases in France, the so-called "French paradox".[1] It is also a potential cancer chemopreventive agent.[2] We found recently that simple structural modification of resveratrol could significantly enhance its antioxidant activity.[3] We report herein that the antioxidant activity of these compounds correlates well with their cytotoxic and apoptotic activities against human leukemia (HL-60 and Jurkat) cell lines. 3,4-DHS and 3,4,5-THS which possess *ortho*-diphenoxyl functionality are the most active antioxidative and cytotoxic ones. The mechanism and implication of this correlation will be discussed.



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Oxiscore, a new proposed summary index to assess age and gender related oxidative status in healthy subjects

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Oxidative stress has been related to several pathologies and to the physiological aging processes. Up to now, no ideal biomarker for oxidative damage is available and studies on age and gender related changes in humans' antioxidants led to conflicting results Objective: This study was performed to generate an index of oxidative stress (Oxiscore) reflecting both oxidative injuries and antioxidant defenses in vivo Methods: We enrolled, and stratified by age and gender, 87 healthy subjects (51 males; age 24-77 years). The free and total malondialdehyde (F-, T-MDA), glutathione disulphide/reduced form ratio (GSSG/GSH) and isoprostane (iPF2α-III) were evaluated and computed in a damage score. Reduced glutathione, α- and γ-tocopherol (TH) and individual antioxidant capacity (IAC), were computed in a protection score. The Oxiscore resulted by subtracting the protection score from the damage score Results: The Oxiscore was significantly increased with ageing ($p = 0.007$) and in male gender ($p = 0.01$). Among the single parameters only MDA was correlated with age ($p = 0.02$) but was insensitive to gender. In relation to gender the only correlate parameters were the GSSG/GSH ratio ($p = 0.002$) and α-TH ($p = 0.005$) Conclusions: Single parameter can only partially define the redox status. The Oxiscore, representing both antioxidant systems and oxidative damage, better describes the age and gender related oxidative stress state in healthy subjects.

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