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PL1

Delaying (or Accelerating) the Degenerative Diseases of Aging

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Delaying (1,2,3). Mitochondrial decay appears to be a major contributor to aging and associated degenerative diseases. Aging mitochondria exhibit a decrease in membrane potential, respiratory control ratio, cardiolipin, and cellular oxygen consumption, and an increase in oxidant by-products. Oxidative damage to DNA, RNA, proteins, and lipids in mitochondrial membranes is a major consequence of this decay, resulting in functional decline of mitochondria, cells, and organs such as the brain. Feeding the mitochondrial metabolites acetyl carnitine and lipoic acid to old rats rejuvenates the mitochondria and improves brain and other function.
Accelerating (4,5). Inadequate dietary intakes of vitamins and minerals are widespread, most likely due to excessive consumption of energy-rich, micronutrient-poor, refined food. Inadequate intakes may result in chronic metabolic disruption, including mitochondrial decay. Deficiencies in many micronutrients cause DNA damage, such as chromosome breaks, in cultured human cells or *in vivo*. Some of these deficiencies also cause mitochondrial decay with oxidant leakage and cellular aging and are associated with late onset diseases such as cancer. I propose DNA damage and late onset disease are consequences of a triage allocation response to micronutrient scarcity. Episodic shortages of micronutrients were common during evolution. Natural selection favors short-term survival at the expense of long-term health. I hypothesize that short-term survival was achieved by allocating scarce micronutrients by triage, in part through an adjustment of the binding affinity of proteins for required micronutrients. If this hypothesis is correct, micronutrient deficiencies that trigger the triage response would accelerate cancer, aging, and neural decay but would leave critical metabolic functions, such as ATP production, intact. Evidence that micronutrient malnutrition increases late onset diseases, such as cancer, is discussed. A multivitamin-mineral supplement is one low-cost way to ensure intake of the Recommended Dietary Allowance of micronutrients throughout life.

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PL2

Nutritional photoprotection of skin : carotenoids and flavonoids

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The concept of systemic photoprotection by dietary means is gaining momentum. Skin is continuously exposed to UV radiation, the major cause of skin disorders such as sunburn, photodamage and non-melanoma skin cancer. Most of the erythemal annual UV dose is encountered under non-vacation conditions, when no sunscreen is applied. In the absence of topically added compounds, skin protection depends solely on endogenous defense. Micronutrients can act as UV absorbers, as antioxidants, or can modulate signaling pathways elicited upon UV exposure. UV-induced erythema is a suitable parameter to assess photoprotection. Dietary protection is provided by carotenoids, tocopherols, flavonoids, and other micronutrients, contributing to maintenance resistance as part of life-long protection. Our work on lycopene (e.g. from tomato) and on flavonoids (polyphenols e.g. from cocoa) will be presented in terms of molecular mechanisms and applicability to human volunteers.

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PL3

Mechanisms of formation and assessment of oxidatively generated damage to DNA

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Major progress has been made during the last decade in the elucidation of oxidative degradation pathways of purine and pyrimidine bases of isolated DNA and related model compounds. More recently attempts were made to validate the occurrence of these chemical reactions in a cellular environment. For this purpose the formation of dedicated oxidized nucleosides was monitored as hallmarks of specific decomposition patterns. Therefore using the accurate and sensitive HPLC-MS/MS assay, 10 single modified nucleosides and bases of the 80 oxidation compounds identified so far in model studies were accurately detected in the nuclear DNA of human cells. Thus, several $^{\bullet}$OH-mediated oxidation pathways of thymine, guanine and adenine were assessed. Evidence was provided for the overwhelming formation of 8-oxo-7,8-dihydroguanine (8-oxoGua) in the DNA of cells exposed to high intensity 266 nm laser pulses. This was rationalized in term of initial bi-photonic ionization of the pyrimidine and purine bases of DNA, followed by hole migration to guanine bases acting as sinks. Another example of oxidative modifications to cellular DNA is provided by the formation of 8-oxoGua in the DNA of cells and human skin upon exposure to UVA radiation as mostly the result of singlet oxygen oxidation. Recent findings show the critical role played by radical oxidation of the sugar moiety at C4 that leads to strand cleavage together with the generation of DNA interstrand cross-links representing the first examples of identified oxidative clustered DNA damage.

OP1

Nutrition, oxidative damage and repair of DNA

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The levels of oxidatively damaged DNA in cells and tissues from animals and humans are a function of ongoing damage and repair. Nutritional factors may modify these levels by altering the damage or repair rate. In this context oxidative damage to DNA bases has mainly been studied as purine modification in terms of 8-oxodG and FPG (formamidopyrimidine glycosylase) sensitive sites or oxidized pyrimidines as endonuclease III sensitive sites in peripheral blood mononuclear cells (PBMC) in human studies. The related repair capacity can be studied in terms of genotypes or expression of the relevant enzymes such as *OGG1, NEIL1, NUDT1*and *MUTYH* at the mRNA level, whereas the activity of OGG1 can be assessed as incisions of oxidized purines. Excreted repair products have been measured in urine. The full interpretation of all these biomarkers awaits validation of their association with cancer risk in prospective settings (Loft & Møller 2006).
A survey of the literature indicates that ingestion of antioxidants may be associated with reduced level of DNA damage in PBMC and urine of humans, albeit the effect is lower than previously expected (Møller & Loft 2006). Part of such effects may be due to enhanced repair, in particular in subjects with compromised nutritional status. In animal models oxidative stress and DNA damage may lead to enhanced repair, whereas such mechanisms have yet to be demonstrated convincingly in humans.
It is important to consider repair capacity when studying interventions or exposures with possible effects on oxidative damage to DNA in living organisms.

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OP2

Mechanisms of protein tyrosine nitration in hydrophilic and hydrophobic biocompartments

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Protein tyrosine nitration is a post-translational modification found *in vivo*, secondary to excess formation and reactions of nitric oxide-derived oxidants. In addition to its possible effects on protein structure and function, tyrosine nitration is being revealed as a biomarker and even a risk factor in a variety of disease conditions including cardiovascular, inflammatory and neurodegenerative disorders. Formation of protein 3-nitrotyrosine depends on free radical mechanisms and, importantly, nitration yields are responsive to increases of either superoxide/hydrogen peroxide or nitric oxide levels; however, nitration yields *in vivo* are typically low, mainly due to the presence of strong reducing systems (*e.g.* glutathione), that can potently inhibit at different levels the nitration process. Evidence is provided to show that the combined existence of metal-catalyzed processes, assistance of alternative nitration steps and favored nitration in hydrophobic environments, provide individually and or in combination, feasible mechanisms of nitration in complex biological milieu. Recent studies using hydrophobic tyrosine analogs and tyrosine-containing peptides have revealed that factors controlling nitration in hydrophobic environments such as biomembranes and lipoproteins can differ to those in aqueous compartments. In particular, exclusion of key soluble reductants from the lipid phase will more easily permit nitration while lipid-derived radicals are suggested as important mediators of the one-electron oxidation of tyrosine to tyrosyl radical in proteins associated to hydrophobic structures. Development and testing of hydrophilic and hydrophobic tyrosine probes that can compete with endogenous constituents for the nitrating intermediates provide not only tools to trace nitration processes and unravel nitration mechanisms *in vitro* and *in vivo*, but also serve cell and tissue protective functions against the toxic effects of protein tyrosine nitration.

OP3

Oxidized protein repair by the methionine sulfoxide reductase system

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The accumulation of oxidatively modified protein is a hallmark of aging. This accumulation results, at least in part, from the increase of reactive oxygen species coming from both cellular metabolism and external factors including environment, but the efficacy of protein maintenance systems is also involved. Since protein oxidation is associated with a loss of function, oxidized proteins must be eliminated from the cells by these protein maintenance systems, through either degradation or repair. Oxidized protein degradation is mainly achieved by the proteasomal system in cytosol and nucleus and it is now well established that proteasomal function is generally impaired with age. Repair is limited to few modifications, such as methionine oxidation, that can be reversed by the peptide methionine sulfoxide reductase enzymes. These enzymes are composed of MsrA and MsrB, which reduce the two diasteroisomers of the oxidized methionine, methionine-S-sulfoxide and the methionine-R-sulfoxide, within proteins, respectively. We reported that peptide methionine sulfoxide reductase activity as well as gene and protein expression of MsrA are decreased in various organs as a function of age. More recently, we have shown that gene expression of both MsrA and MsrB2 (CBS-1) is decreased during replicative senescence of WI-38 fibroblasts, and this decline is associated with an alteration in catalytic activity and the accumulation of oxidized protein. To analyze the relationship between oxidative stress, protein oxidative damage and Msr, MsrA full-length cDNA has been overexpressed in SV40 T antigen-immortalized WI-38 human fibroblasts and MsrB2 full-length cDNA has been overexpressed in Molt-4 lymphoblastoid cells. After hydrogen peroxide-induced oxidative stress, both MsrA- and MsrB-overexpressing cells exhibit lower protein oxidative damage than control cells. These results indicate that the Msr system may play an important role in cellular defenses against oxidative stress by protecting proteins against oxidation and limiting the accumulation of oxidized proteins.

OP4

600g of fruits and vegetables in everyday life : repercussion on plasma antioxidants and markers of oxidative stress

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Epidemiological studies indicate that increased consumption of fruits and vegetables is associated with a decreased risk of chronic degenerative diseases including cardiovascular diseases and cancer. Such a positive effect is expected (but not really proved) to be correlated with the high antioxidant content of fruits and vegetables. If health professionals do advise the intake of at least 5 daily servings of fruits and vegetables (the 5 – a – day concept) for a better health, several questions are, however, opened:
1° what do really mean 5 servings regarding to the ingested amount?
2°do low fruit/vegetables eaters have decreased antioxidant defences than those eating the famous 5 servings, and if yes, in which proportion? The ELAN study (Etude Liégeoise sur les ANtioxydants performed in Liège, Belgium) performed on 868 healthy volunteers will try to give some answer to this question (see poster).
3° has an enriched fruits and vegetables diet really a direct impact on the antioxidant defences? Is it also able to decrease biomarkers of oxidant damage associated with disease risk?
4° which compounds are involved in the antioxidant capacity of fruits and vegetables?
5° are plasma vitamin C and ? – carotene useful markers of high intake of fruits and vegetables?

OP5

Effect of exercise and obesity on oxidant-antioxidant status in relation with IL-6 in adolescent girls.

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It is now well established that the pubertal period is associated with insulin resistance and in girls with fat gain. At the same time, in most of the industrialized countries, a severe decrease in physical activity often occurs which tends to track throughout life. Thus adolescent girls are at high risk of obesity, a chronic disease characterized by insulin resistance and a low-grade inflammation level, 2 factors well-known to induce an imbalance between oxidant and antioxidant status. Acute and intense exercise is another potent factor well known to increase the risk of oxidative stress. Nevertheless, it has been also demonstrated to induce a muscular secretion of myokines, such as IL-6 which acts paradoxically as an anti-inflammatory factor. Currently no information are available about the IL-6 release at exercise in young obese both by the muscle and the adipocyte. Thus the question arises about the resulting effect on the oxidant-antioxidant status at exercise.

So, we studied the respective oxidant-antioxidant blood status in relation with IL-6, at rest and after maximal exercise, in healthy (C) and overweight or obese lebanese adolescent girls (O). We were able to demonstrate that fat gain at this period is associated with a decrease in plama Vitamin E/cholesterol and β-carotene/cholesterol and with alterations in the erytrocyte GPx activity and GSH/GSSG ratio. Exercise results in the same IL-6-increase in both groups. Whereas exercise fails to induce any significant oxidative stress in C, it results in a significant increase of oxidative stress biomarkers (MPO, ROOH) in O. All these data are discussed in relation with rest antioxidant status, physical fitness (VO_2peak), energetic expenditure, and exercise-IL-6 increase.

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OP6

Vitamin C jeopardizes training efficiency in mice and men. When should we give antioxidants to our athletes?

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Physical exercise is a double-edged sword. If practised in moderation it has considerable health advantages1. If, however, practised to exhaustion it may cause damage to muscle, joints, and other tissues. Aware of this, exercise practitioners take supplements of many kinds. It is estimated that 70% of the US population use dietary supplements at least occasionally and 40% uses them on a regular basis. Antioxidant vitamins are amongst the most widely used dietary supplements taken in exercise. One of the most commonly used is vitamin C. There is considerable debate regarding the long term beneficial health effects of vitamin C supplementation. We have tested the effect of vitamin C supplementation on training efficiency in both rats and humans.

The human study was double blind and randomized. We trained 14 subjects for 8 weeks. Five subjects took 1g of vitamin C daily. In the animal study, 36 male Wistar rats were exercised for 3 or 6 weeks. Twelve animals were treated daily with vitamin C (0,35 mg/cm^2 of body surface). Training was estimated measuring running capacity to exhaustion (in rats) or increases in VO_2max (in persons). The effect of training on muscle mitochondrial biogenesis and antioxidant enzyme expression was also measured.

We found that in persons, 8 weeks of training increased VO_2max by 22% ($p<0.05$). However, in the group that took vitamin C the increase was non significant. Untrained rats ran for 100 minutes and after 6 weeks of training they ran for 300 minutes, but the group of rats treated with vitamin C ran for 120 minutes only. We offer a molecular explanation for this, that vitamin C decreases exercise-induced expression of transcription factors involved in mitochondrial biogenesis and of markers of mitochondrial content. It also prevents the increase in the expression of antioxidant enzymes such as SOD or GPx which occurs after training.

Vitamin C supplementation decreases training efficiency in humans and in animals because it prevents cellular adaptations to exercise.

OP7

Mechanism of a -tocopheryl-phosphate (a-TP) transport across the cell membrane

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We have reported that a-TP is synthesized and hydrolyzed in animal cells and tissues; it modulates also several cell functions (FRBM 39:970, and UBMB Life, 57:23, 2005). While it is similar to a-tocopherol (a-T), a-TP appears to be more potent than a -T in inhibiting cell proliferation, down regulating CD36 transcription, inhibiting atherosclerotic plaque formation etc. In cells and animals a -TP does not act by liberating a-T; rather, the intact molecule appears to be more potent than a -T itself (BBRC, 318:311, 2004; ABB, 450:63, 2006). Administration of a-TP to cells or to animals requires its transfer through membranes, an event that cannot occur by simple diffusion due to the size and the charge of the molecule but requires a transporter. The inhibitory effect of a-TP on the proliferation of THP-1 was used as an indication of a -TP transport through the cell membrane. Specific transport inhibitors, glibenclamide and probenecid showed no inhibition of cell proliferation or cytotoxicity. However, both compounds prevented, dose-dependently, a-TP inhibition of cell proliferation. The data indicate that a-TP enters cells via a glibenclamide- and probenecid-sensitive transport system. Both, members of the ABC transporter family and of the organic anion transporters (OAT), appear to be sensitive to these two inhibitors. However, since ABC transporters function to export cell solutes and a-TP is imported, a-TP transport may occur *via* an OAT family member (supported by USDA agreement No. 581950-9-001 and Novartis and Phosphagenics fellowships to YN).

OP8

Cytosolic redox signaling and mitochondrial function through protein post-translational modifications

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Mitochondria generate second messengers, such as H_2O_2 and nitric oxide ($^{\cdot}NO$), which are involved in the regulation of redox-sensitive cell signaling through the mitogen-activated protein kinase (MAPK; *e.g.,* JNK) pathway, thus coordinating functional responses between mitochondria and other cellular processes. Conversely, mitochondria are the recipients of cytosolic signaling molecules, such as JNK, which translocates to mitochondria under stress conditions and during aging and elicits profound metabolic effects in the organelle through the activation of phosphorylation cascades. The interaction between these two processes establishes a regulatory device that controls cellular energy levels and redox environment. Impairment of the communication between mitochondrion-supported redox signaling and cytosolic signaling pathways may be the basis for the mechanisms inherent in cell death pathways and the loss of cell function associated with aging and age-related degenerative disorders.

The regulatory mechanisms involve mitochondrial protein post-translational modifications, including phosphorylation, tyrosine nitration, and cysteine S-nitrosation. JNK, phosphorylated and activated was shown to translocate to the mitochondrial outer membrane and induce a signaling pathway that leads to the phosphorylation and inhibition of mitochondrial matrix pyruvate dehydrogenase as well as the phosphorylation of mitochondrial outer membrane apoptosis-related proteins, Bcl-2/Bcl-x_L, and the release of cytochrome c. During oxidative/nitrosative stress, GSSG and GSNO (S-nitrosoglutathione) accumulate and modulate protein functions by post-translational modifications, *i.e.*, glutathionylation and S-nitrosation, respectively. GSNO from the cytoplasmic space was shown to induce cysteine S-nitrosation of mitochondrial ANT (adenine nucleotide translocase), VDAC (voltage-dependent anion channel), and ATP synthase F_1 complex α subunit. The latter modification inhibited the ATPase activity and potentiated the Ca^{2+}-sensitive opening of the mitochondrial permeability transition pore. Glutathionylation of ATP synthase and aconitase was shown to inhibit their activities. Accumulation of nNOS was detected during aging and upon inhibition of 26S proteasome activity, and is accompanied by an increase of mitochondrial protein tyrosine nitration, which was shown to inhibit aconitase activity. From these findings, it may be surmised that post-translational modifications of mitochondrial proteins play an important role in the determination of cell fate by regulating critical mitochondrial functions, including apoptosis and energy metabolism. A complex crosstalk and interplay of cytosolic signaling pathways converge on the mitochondria, which ultimately function as an epicentral processor, aside from the nucleus, to determine the death/survival of the cell.

OP9

ROS signaling: Thiol-based peroxidases as H_2O_2 sensors and redox transducers

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The ROS biological paradox of being both toxic and acting as signalling molecules is a puzzling question, which underlies mechanisms important for the integrity and fitness of living organisms and their aging. The pathways regulating ROS homeostasis provide both strong evidence of ROS signalling specificity and mechanisms mitigating ROS toxicity. By taking advantage of the chemistry of ROS, nature has evolved highly specific mechanisms that form the basis of oxidant scavenging and ROS signalling systems. We will present the physiology and molecular mechanisms of ROS homeostatic pathways in the yeast *S. cerevisiae* (Orp1-Yap1) and *S. pombe* (Tpx1-Pap1), which can be assimilated to H_2O_2 receptors that share a common mechanism. Their regulatory component, the Yap1 and Pap1 transcription factors, are activated by reversible disulfide bond formation. H_2O_2-induced oxidation of Yap1 and Pap1 is not direct involving respectively the thiol-based peroxidases Orp1, a GPx-like enzyme, and Tpx1, a peroxiredoxin, which relay the peroxide signal by means of a thiol-oxidation cascade. Orp1 and Tpx1 are thus peroxide receptors and redox transducers. Pap1 but not Yap1 activation is restricted within a narrow range of H_2O_2 concentration. This is due to Tpx1 oxidation to an inactive cysteine-sulfinic acid form, eventually reversed by ATP-dependent reduction by sulfiredoxin. These mechanisms illustrate the built-in high specificity of cysteine-based redox regulation and suggest the existence of specific pathways of cysteine oxidation.

OP10

NF-?B activation by Reactive Oxygen Species: fifteen years later

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The transcription factor NF-?B plays a major role in coordinating innate and adaptive immunity, cellular proliferation, apoptosis and development. Since the discovery in 1991 that NF-? B may be activated by H_2O_2, several laboratories have put a considerable effort into dissecting the molecular mechanisms underlying this activation. Whereas early studies revealed an atypical mechanism of activation, leading to I?Ba Y42 phosphorylation independently of I?B Kinase (IKK), recent findings suggest that H_2O_2 activate NF-?B mainly through the classical IKK-dependent pathway. The molecular mechanisms leading to IKK activation are, however, cell-type specific, and will be in our talk. We will also describe the effect of other ROS (HOCl and $^1O_2$) and Reactive Nitrogen Species on NF-?B activation. Finally, we critically review the recent data highlighting the role of ROS in NF-?B activation by proinflammatory cytokines (TNF-a and IL-1ß) and lipopolysaccharide (LPS), two major components of innate immunity.

OP11

Selenium and sulfur-dependent enzymes in defense against oxidative stress

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Thioredoxin, which is reduced by NADPH catalyzed by thioredoxin reductase, and glutaredoxin, which is reduced by glutathione, have overlapping activities as reductases *in vivo*. Mammalian cytosolic thioredoxin reductase (TrxR1) is a selenoprotein with a broad substrate specificity and a -Gly-Cys-Sec-Gly active site, where Sec is selenocysteine. Reduced TrxR1 is inhibited by arsenic trioxide. The enzyme is targeted by many electrophilic compounds including curcumin. Derivertized TrxR1 enzymes are inactive as thioredoxin reductases, but may also show enhanced NADPH oxidase activity. The activity of mammalian TrxR1 is decreased in most tissues byselenium deficiency. We have used rats, fed a selenium-deficient diet for 13 or 52 weeks and studied liver, kidney and brain cytosol fractions along with controls. In selenium deficient animals, the activity of thioredoxin reductase fell most in liver, followed by kidney. It did not decrease in brain. The mRNA for TrxR1 did not decrease in liver. Liver cytosolic TrxR1 was examined for selenium deficiency-related alterations. No evidence was obtained for truncated protein. Instead, full length thioredoxin reductase was found in fractions and the structure of the modified enzyme will be described. Selenium deficiency did not alter the levels of glutaredoxin and glutathione activity in liver tissue. The unique structure of thioredoxin reductase with its essential selenocysteine residue located in a unique position will be discussed in relation to oxidative stress defense.

OP12

Inhibition of the selenoenzyme thioredoxin reductase by gold (I/III) complexes alters cellular redox balance and drives the cell to apoptosis

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Several metals are essential for biological functions and some are used in medicine as drugs or diagnostic agents. Gold compounds, utilized over the years for the treatment of rheumatoid arthritis, are now recognized as promising antitumor agents. Gold(I/III) derivatives, with different ligands forming either linear two-coordinate or square planar complexes, are potent inhibitors of both cytosolic and mitochondrial thioredoxin reductase activity. This inhibition appears to depend on the interaction of the gold ions with the selenolate moiety of the enzyme that acts as a soft base. In isolated mitochondria gold complexes are shown to induce permeability transition, loss of membrane potential and release of cytochrome c. All these events are accompanied by a marked stimulation of reactive oxygen species (ROS) production. On the contrary, respiration and glutathione redox state are not altered by gold compounds. Increased ROS formation was also measured in cultured cells treated with gold(I/III) compounds that also decrease viability and stimulate apoptosis observed as cytochrome c release, caspase activation and DNA fragmentation.
It is proposed that gold(I/III) compounds, acting as potent inhibitors of thioredoxin reductases, determine the alteration of cellular redox state due to an increased concentration of hydrogen peroxide and oxidized thioredoxin. These conditions are the determinant of cell apoptosis that appears to depend both on the increase of mitochondrial membrane permeability and on the stimulation of the mitogen activated protein kinases (MAP kinase) system.

OP13

Insight from GPxs structure and function

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The multigene family of GPxs encodes for tetrameric and monomeric enzymes both containing either a selenocysteine (Sec) or cysteine (Cys) residue at the catalytic site. Monomeric GPxs carry deletions of the subunit interfaces. The Sec containing homologues are found in vertebrates, and, with a scattered presence, in lower organisms. These SecGPxs are either tetrameric or monomeric, as the mammalian GPx-1 and 4 respectively, and undergo a peroxidatic cycle where the selenenic acid derivative of Sec (SeOH), formed upon reaction with the peroxide, is reduced back at a fast rate by glutathione (GSH). On the other hand, terrestrial plants, insects, bacteria, fungi, do not encode for any SecGPxs, but for CysGPxs only. By analyzing more than 450 GPx sequences, we have found that the majority of these both carry a non-aligned 'second' Cys residue in the fourth helix and have a monomeric nature, being, in the latter respect, GPx-4 homologues. In these CysGPxs, the oxidized intermediate of the catalytic cycle is different from SecGPxs, being accounted for by a disulfide between the peroxidatic Cys and the 'second' Cys, thus acting as a 'resolving' Cys, as typically in peroxiredoxins. Conformation analysis suggests that the absence of tetrameric structure allows the loop flexibility required for the disulfide formation. Taking as a paradigm the *Drosophila* CysGPx, we have shown that the above features largely favor the reaction with Thioredoxin (Trx) over GSH. Thus, the majority of the CysGPxs sequences deposited in the data banks, in functional terms, must be referred to as Trx peroxidases. On the other hand, the functional role of the quantitatively minor tetrameric or monomeric CysGPxs sequences, lacking the resolving Cys, which, in vertebrates, are coexisting together with the SecGPxs, remains unresolved. GPxs are not redundant proteins. In yeast one of the above CysGPxs, GPX3, has been shown to be involved in the peroxide-dependent activation of the trascription factor Yap-1 (Toledano *et al. TIBS* 29, 351, (2004). While in mammals, GPx-1 appears the unique, real, glutathione peroxidase out of the five SecGPxs, the others might have different functions. In particular, GPx-4 is involved in the oxidation of protein thiol motifs taking place during sperm maturation.

OP14

Vitamin E and vesicular transport

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Vitamin E has for long been considered to be an antioxidant only, However, evidence is accumulating that this can not be its major physiological function. Apart from influencing signaling cascades, vitamin E is able to influence gene activity. To find a most comprehensive list of α-tocopherol-regulated genes, the global gene ex-pression profiles of livers from mice, fed diets different in α-toco-pherol content, were compared using DNA microarray technology. 389 genes were changed in the supplemented diet group compared to the deficient group, 296 transcripts were up-regulated and 93 down-regulated. Functional clustering using the EASE bioinfor-matics software package identified a group of 121 genes involved in transport processes. 21 of these genes, from which 19 were up-regulated by α-tocopherol, are specifically involved in (synaptic) vesicular trafficking. The up-regulation of syntaxin 1C (*Stx1c*), vesicle-associated membrane protein 1 (*Vamp1*), N-ethyl-maleimide-sensitive factor (*Nsf*) and syntaxin binding protein 1 (*Stxbp1, Munc18-1*) was verified by real time PCR. At a functional level, α-tocopherol increased the secretory response in RBL and PC12 cells. Although here detected in liver, most of the α-toco-pherol-responsive transport pathways are also relevant to neuro-transmission. A role of α-tocopherol in the vesicular transport might be related to the neural dysfunctions observed in severe α-tocopherol deficiency.

OP15

Targeting Inflammation for Prevention and Treatment of Cancer: Food For Thought

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Dietary agents have been linked with prevention and therapy of cancer through a mechanism that is not well understood. We postulate that inflammation plays a major role in tumorigenesis through the activation of NF-kB. We also postulate that dietary agents mediate their effect through modulation of NF-kB activation. NF-kB, a transcription factor, is present normally in the cytoplasm as an inactive heterotrimer consisting of p50, p65 and IkBa subunits. When activated, NF-kB translocates to the as a p50-p65 heterodimer. This factor regulates the expression of various genes that control apoptosis, viral replication, tumorigenesis, various autoimmune diseases, and inflammation. NF-kB has been linked to the development of carcinogenesis for several reasons. First, various carcinogens and tumor promoters have been shown to activate NF-kB. Second, activation of NF-kB has been shown to block apoptosis and promote proliferation. Third, the tumor microenvironment can induce NF-kB activation. Fourth, constitutive expression of NF-kB is frequently found in tumor cells. Fifth, NF-kB activation induces resistance to chemotherapeutic agents. Fifth, several genes involved in tumor initiation, promotion, and metastasis are regulated by NF-kB. Sixth, various chemopreventive agents have been found to downregulate the NF-kB activation. All these observation suggest that NF-kB could mediate tumorigenesis and thus can be used as a target for chemoprevention and for the treatment of cancer. Besides NF-kB, we have also targeted AP-1 and STAT3, other transcription factors that mediate tumorigenesis. We will present the data that shows that phytochemicals derived from fruits, vegetables and spices are important inhibitors of NF-kB activation, and can suppress the expression of genes involved in carcinogenesis and tumorigenesis *in vivo*.

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OP16

Macronutrient intake induces oxidative and inflammatory stress while insulin causes suppression of ROS generation and inflammation: relationship to the metabolic syndrome

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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)[1], we have shown that glucose, equicaloric amounts of fat (eaten as cream)[2] and a mixed fast food meal (900 calories) [3] 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 NFkB binding, a fall in IkBa expression and an increase in IKKa and IKKb expression. There is a concomitant increase in TNFa mRNA in the MNC[4]. 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, and tissue factor (TF)[5] . 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. In addition, there is a significantly greater and more prolonged ROS generation and NFkB binding following macronutrient intake in the obese when compared with normal subjects. 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[6]. Plasma TNFα[7] and CRP concentrations also fall following caloric restriction and weight loss. A 48 hour fast in normal subjects also leads to a reduction in ROS generation by 50% and a parallel reduction in p47phox, orthotyrosine and metatyrosine[8]. 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 NFkB binding with an increase in IkBa expression[9,10]. In addition, there is a suppression of AP-1 and Egr-1, MMP-2, MMP-9, PAI-1 and tissue factor (TF) [11]. The anti-inflammatory effect of insulin was further confirmed in patients with acute myocardial infarction who were treated with a low dose insulin infusion in addition to the standard thrombolytic therapy. Insulin infusion led to a significant fall in C-reactive protein (CRP), serum amyloid (SAA), PAI-1, MMP-1 and oxidative stress[12]. In addition, insulin had a significant suppressive effect on the increase in plasma CK, CKMB and myoglobin concentrations in these patients, consistent with a cardioprotective action. This effect of insulin on CRP and SAA has now been confirmed both in acute myocardial infarction and in patients undergoing coronary artery bypass surgery. These facts allow us to conclude that there exists a novel relationship between macronutrient intake and insulin, the hormone secreted in response to macronutrient intake. This relationship extends beyond the classical paradigm involving metabolic mechanisms only. It encompasses oxidative and inflammatory stress following macronutrient intake and the suppression of these processes with insulin, the hormone which is secreted in response to macronutrient intake.

We are now engaged in (1.) 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; (2.) The use of insulin as an anti-inflammatory, cardioprotective and neuroprotective agent in acute myocardial infarction and stroke. The

metabolic syndrome may be reinterpreted in light of these observations as a pro-inflammatory consequence of excessive macronutrient intake, inflammation and insulin resistance[13].

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OP17

Hypercholesterolemia related changes in rabbit aorta and brain : protection by alpha tocopherol

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Hypercholesterolemia, a major risk factor for age related diseases such as atherosclerosis and Alzheimer's disease (AD). Changes in human plasma cholesterol levels results from the interaction between multiple genetic and environmental factors. The accumulation of excess cholesterol in blood vessels leads to atherosclerosis. Studies show that differential expression of oxidative stress proteins, lipid metabolism related enzymes and receptors response to atherogenic diet. Excess brain cholesterol has been associated with increased formation and deposition of amyloid-β peptide from amyloid precursor protein which may contribute to the risk and pathogenesis of AD. More than 50 genes have been reported to influence the risk of late-onset AD. Several of these genes might be important in cholesterol metabolism and transport.

On the basis of these results, an *in vivo* study has been carried out. Rabbits were fed with cholesterol supplemented diet or cholesterol supplemented diet plus alpha tocopherol, after 4 weeks aortas and brains were removed. Atherosclerotic index calculated from the lesions stained by Sudan V. Protein kinase C and scavenger receptor expression was measured in aortic pieces. On the other hand proteasome function, protein carbonylation, tau hyperphoshorylation and amyloid-ββprotein evaluated in the brain of hypercholestrolemic rabbits. The results indicate a cellular mechanism for hypercholesterolemia induced atherosclerosis and AD similar changes will be discussed.

OP18

Multi-level anti-inflammatory effects of French maritime pine bark extract (Pycnogenol)

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French maritime pine bark extract has been used for inflammatory diseases in traditional medicine and recent clinical studies support anti-inflammatory pharmacological effects. Due to the complexity of the extract composition and the fact that not all extract components are bioavailable it is a challenge to elucidate the molecular pharmacological basis for the observed anti-inflammatory action. Our approach took into consideration that plasma samples after oral intake of the extract would contain any active principles. Therefore, we obtained blood samples before and after five days administration of 200 mg Pycnogenol to healthy humans as well as plasma samples before and after a single intake of 300 mg. Plasma samples were tested in different assays evaluating key mechanisms of inflammation, namely activation of the master molecule of inflammation, NF-? B, activation of the eicosanoid generating enzymes cyclooxygenase 1 and 2 (COX-1 and COX-2) and release of the matrix degrading enzyme MMP-9 (matrix metalloproteinase 9) that contributes to the pathogenesis of various chronic inflammatory diseases. Plasma samples obtained before and after repeated intake of 200 mg Pycnogenol statistically significantly inhibited MMP-9 release from human monocytes and NF-? B activation. Likewise, these plasma samples moderately inhibited both COX-1 and COX-2 activity *ex vivo.* Plasma samples obtained after a single dose of 300 mg induced a statistically significant increase in the inhibition of both COX-1 and COX-2 only 30 min after ingestion of the pine bark extract. This suggests a strikingly rapid bioavailability of bioeffective compounds after oral intake of the extract. To conclude, after oral intake of Pycnogenol human plasma contains sufficient concentrations of active principles to inhibit key mediators of inflammation.

OP19

Inflammation and bowel disease: potential protection by flavanols and procyanidins

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It is accepted that the major actions of ingested flavanol oligomers (procyanidins) are limited to the gut lumen where they could protect the integrity of the epithelium, acting for example, as anti-inflammatory and antioxidant agents. Using cultured Caco-2 cells as a model of intestinal epithelium we investigated the capacity of hexameric procyanidins to interact with cell membranes, and prevent consequent oxidative damage and pro-inflammatory events. A purified fraction of hexamers of epicatechin (Hex) isolated from cocoa was assayed at physiological relevant concentrations. Hex interacted with Caco-2 cell membranes protecting from the selective loss of cell monolayer permeability, and the increase production of cell oxidants. These protective effects were observed when the cells were challenged by either a bile acid or a free radical generator. When cells were subjected to proinflammatory conditions, Hex inhibited tumor necrosis factor alpha (TNF?)-induced cell oxidant increase, and TNF? -induced NF-? B activation. The inhibition of NF-? B activation was observed at different levels of the signaling cascade. In conclusion, dietary procyanidins that are not absorbed at the gut lumen can protect the intestine mucosa from oxidant and pro-inflammatory stimuli. These effects stress the potential capacity of plant flavan-3-ols and derived procyanidins to ameliorate the colonic inflammatory processes associated with inflammatory bowel disease, as well as other pathological conditions.
Mars Incorporated (USA) isolated and characterized the hexamer fraction, and provide partial support.

OP20

Neurotrophic Activities of Flavonoids

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Neurotrophic factors play key roles in the survival, differentiation and functional maintenance of nerve cells. Several years ago, we showed that specific flavonoids such as fisetin (3, 7 ,3', 4' tetrahydroxyflavone) could promote the differentiation of nerve cells in culture suggesting that some flavonoids have neurotrophic activity. To test this hypothesis further, we examined the ability of fisetin to promote nerve cell survival following trophic factor withdrawal and after exposure to oxidative insults. Fisetin proved very effective at preventing nerve cell death in a variety of different cell death paradigms. The neuroprotective actions of fisetin involve both its antioxidant activity as well as its ability to maintain intracellular levels of glutathione, the major intracellular antioxidant. Fisetin also activates the Ras-ERK cascade which is required for its effects on differentiation. Activation of this cascade has also been implicated in memory. To test the idea that fisetin can promote memory, we first looked at its effect on long-term potentiation, the primary model for studying memory in vitro. Low doses of fisetin facilitated long-term potentiation in rat hippocampal slices. Furthermore, using the object discrimination assay, a popular model for studying learning and memory in mice, we showed that oral administration of fisetin could effectively enhance memory. These data indicate that flavonoids such as fisetin have all of the characteristics of neurotrophic factors suggesting that they could be used as therapeutic agents for pathologies in which loss of neurotrophic activity has been implicated. In support of this concept, we have recently found that intravenous administration of fisetin can reduce behavioral deficits in a rabbit stroke model and oral administration can prevent striatal dopamine loss in the MPTP model of Parkinson's disease in mice.

OP21

Role of food association on the *in vivo* antioxidant activity of plant foods.

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The human diet contains an array of plant-derived phytochemicals with antioxidant activity that help the body to cope with oxidative stress. *In vitro* studies indicate that phenolic compounds, widely present in the vegetable kingdom, might play a key role in the protective effect of plant foods. However, a large body of evidences highlight the low degree of absorption of phenolics *in vivo*. The food matrix in which phenolics are ingested might represents a confounding factor could have a major influence on their bioavailability and *in vivo* antioxidant activity. Our group showed that milk addition annihilated the *in vivo* antioxidant effect of tea and chocolate in healthy human subjects. These findings are not without controversy, with other studies showing no effect. In order to investigate the effect of food association on the *in vivo* antioxidant properties of phenolic-rich foods, we set up an intervention study in humans. Eleven healthy volunteers, non-smoking, normo-lipidaemic, taking no supplements and not on any medication, were asked to eat, in a crossover design, 200 g of blueberries with and without milk. On the day of the study, after an overnight fast, basal venous blood samples have been collected prior to feeding and at different time points after food ingestion. Marker of plasma single antioxidants and Total Antioxidant Capacity have been measured. Results shows that milk addition to blueberries have a clear impact on the ability of blueberries to modulate plasma antioxidant capacity and on the absorption of selected phenolics. The possibility that dietary constituents impair *in vivo* antioxidant properties of plant foods cannot be neglected and needs to be further investigated.

OP22

Selenoprotein synthesis and regulation in mammalian cell lines

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Selenium is an essential trace element, which is co-translationally incorporated into selenoproteins as Selenocysteine (Sec), the 21st amino acid. Selenoproteins synthesis follows a remarkable mechanism which involves translational recoding of a UGA codon, normally used as a stop signal, into a selenocysteine insertion signal. In eukaryotes, a specific secondary structure in the 3'UTR of the selenoprotein mRNA is required to direct faithful recoding. The deletion or mutation of this Sec Insertion Sequence (SECIS) results in decoding UGA as a default stop codon. Several components of the eukaryotic Sec insertion machinery have been characterized so far: a Sec-tRNA^{Sec}, an elongation factor (EF-Sec or mSelB), and two SECIS binding proteins (SBP2 and ribosomal protein L30). In our model, SBP2 and L30 perform different functions in the UGA recoding mechanism, with the SECIS acting as a molecular switch characterized by a conformational transition upon protein binding.
Twenty five selenoproteins have been identified in human, among which some are essential for fundamental defense mechanisms against oxidative stress such as glutathione peroxidases (GPx) and thioredoxin reductases (TR). The GPx family is particularly sensitive to selenium regulation and has been mostly studied. In the rat liver, during dietary selenium deprivation, GPx1 (non-essential) activity will be dramatically reduced up to 1%, while GPx4 (essential) is maintained at 75% compared to animals fed with a control diet. Messenger RNA levels can not explain the change in GPx activities and some studies suggest a translational regulation of selenoprotein synthesis. We developped a luciferase-based reporter gene system to define *ex vivo* the precise implications of the SECIS element on the regulation of selenoprotein synthesis (Chavatte *et al.*, 2005 *Nat. Struct. Mol. Biol.*). Firefly luciferase coding region (in which Cys^{258} is mutated into UGA) is linked to various SECIS elements in a mammalian expression vector to assay the UGA-Sec recoding activity in cultured cells. The reporter constructs were transfected into mammalian cells to either transiently or stably express our reporter activity. The influence of various SECIS elements, selenium status of the culture cell media, or oxidant conditions on UGA-Sec recoding activity in different cell lines has been investigated.

OP23

Cigarette Smoke Affect Vitamin E Trafficking via the Modulation of Scavenger Receptor B1

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The lung's delivery systems for tocopherol and other antioxidants remains incompletely understood. Scavenger Receptor B1 (SR-B1) has been shown to play a prominent role in the uptake and delivery of vitamin E (a-tocopherol) from HDL to the cells regulating the vitamin E status of the lung. Furthermore, our in vivo previews studies have suggested (Valacchi et al. ubmitted manuscript) that SR-B1 expression can be modulated by cigarette smoke (CS) exposure.

To further characterize the molecular mechanism (s) involved in this modulation, human airway epithelial cells (A549) were exposed to CS. A549 is an alveolar epithelial cell line that has similarities to Type II cells including similar responses to reactive oxygen species-mediated apoptosis and inhibition of wound repair. A549 cells were fixed in 4% paraformaldehyde, permeabilized and probed with anti SR-B1 and assessed by confocal microscopy. The results showed that in the control, SR-B1 was mainly perinuclear with little present on the cell membrane while in the CS exposed cells (1 cigarette for 45 min) SR-B1 was translocated and localized in large patches on the cell surface membrane. This localization was lost after 24 hours, with a dramatic decline in SR-B1 expression. FACS analysis of membrane SR-B1 using FITC labeled anti SR-B1 confirmed that very low plasma membrane SR-B1, ca 1.2% was present in the control while a marked increase from 20% to 70% at 1 and 2 hrs respectively after CS exposure. Finally the effect of CS on SR-B1 mRNA stability was assessed by adding actinomycin (5mg/ml), mRNA was extracted at 0, 1, 2 , 3 hrs and SR-B1 messenger level was analyzed. Values, expressed relative to the SR-B1 mRNA level for air treated controls (100%), declined similarly between the air and the CS treated cells, indicating that CS had no significant effect on mRNA stability but on SRB1 mRNA expression. Furthermore, an increase of SR-B1 ubiquitination was observed immediately after CS exposure.
These results not only confirm our previous in vivo data that showed reduction in SR-B1 in the lungs of CS- exposed mice, but also suggest that SR-B1 post-translational mechanism(s), ie, sub-cellular localization, could modulate tocopherol transport and trafficking in response to CS (as well as other oxidant assaults) in lung epithelial cells.

OP24

Oxidative stress due to opening of a mitochondrial palmitate+Ca^{2+}-activated, cyclosporin A-insensitive pore.

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Abstract
There are some health problems associated with palmitate (Pal) – the anion of the 16C saturated fatty acid. Ca^{2+} is known to activate phospholipase A_2 also intracellularly with liberation of fatty acids, including Pal. Ca^{2+} is also a well-known promoter of the mitochondrial permeability transition (MPT), which is due to the opening of a cyclosporin A (CsA)-sensitive pore in the mitochondrial inner membrane. This is promoted by oxidative stress and causes swelling of mitochondria, permeabilization of the inner membrane to solute <1.5 kDa, uncoupling and loss of the membrane potential ($\Delta\psi$) and release of cyt c and other proapoptotic factors. We have described a similar system due to the opening of a CsA-insensitive pore by a complex of Pal with Ca^{2+} (PalCaP). The opening of the PalCaP may be reversible under some conditions [Mironova *et al*., J. Bioenerg. Biomembr. (2004), 36, 171-178]. Here we describe the PalCaP in more detail. Pal may form certain membrane domains, which promotes pore formation with Ca^{2+}. There may be a prolonged depolarization of $\Delta\psi$, release of Ca^{2+} and swelling of mitochondria like in MPT. Ruthenium red and La^{3+} – inhibitors of the uniporter – promote repolarization and inhibit the release of divalent cations taken up. Ca^{2+} is thus needed in the matrix for the formation of the PalCaP. The operation of the Ca^{2+}-cycle due to uptake by the uniporter and release by PalCaP can be observed in the presence of increased amounts of fatty acids due to activation of phospholipase A_2 under some pathological conditions.

OP25

A Novel Mechanism of Metal-independent Decomposition of Organic Hydroperoxides and Formation of Alkoxyl Radicals by Halogenated Quinones

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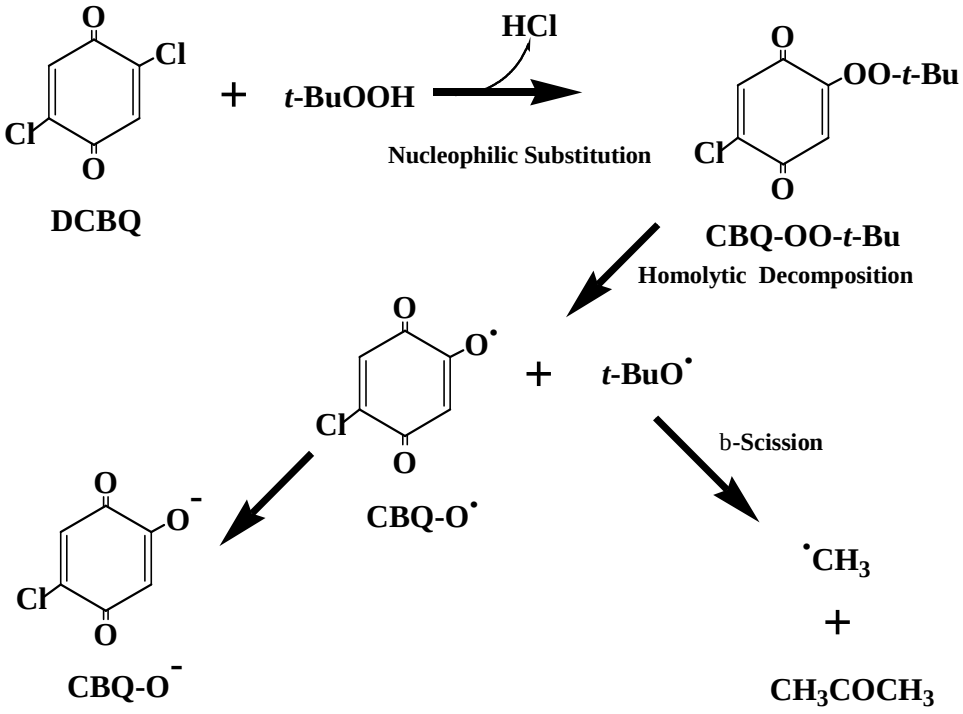
The metal-independent decomposition of organic hydroperoxides and the formation of organic alkoxyl radicals in the absence or presence of halogenated quinones were studied with electron spin resonance (ESR) and the spin-trapping agent 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). We found that 2,5-dichloro-1,4-benzoquinone (DCBQ) markedly enhanced the decomposition of *tert*-butylhydroperoxide (*t*-BuOOH), leading to the formation of the DMPO adducts with *t*-butoxyl radicals (*t*-BuO$^{\bullet}$) and methyl radicals ($^{\bullet}CH_3$). The formation of DMPO/*t*-BuO$^{\bullet}$ and DMPO/$^{\bullet}CH_3$ was dose-dependent with respect to both DCBQ and *t*-BuOOH, and was not affected by iron-specific or copper-specific metal chelators; Interestingly, their formation was markedly inhibited by a variety of antioxidants such as ascorbate and dihydrolipoic acid. Comparison of the data obtained with DCBQ and *t*-BuOOH with those obtained in a parallel study with ferrous iron and *t*-BuOOH strongly suggested that *t*-BuO$^{\bullet}$ was produced by DCBQ and *t*-BuOOH through a metal-independent mechanism. Other halogenated quinones were also found to enhance the decomposition of *t*-BuOOH and other organic hydroperoxides such as cumene hydroperoxide, leading to the formation of the respective organic alkoxyl radicals in a metal-independent fashion. The reaction intermediate and final products between DCBQ and *t*-BuOOH were identified by electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS). The mass spectrum of DCBQ in CH_3COONH_4 buffer (pH 7.4, 0.1 M) is characterized by a two-chlorine isotope cluster at *m/z* 176 and a small one-chlorine isotope cluster at *m/z* 157. The addition of *t*-BuOOH to DCBQ led to complete disappearance of the molecular ion peak cluster at *m/z* 176, significant increase of the intensity of peak cluster at *m/z* 157, and appearance of two new one-chlorine isotope clusters at *m/z* 229 and 172. Tandem mass spectrometric analysis showed that the peak at *m/z* 229 is unstable and can be readily fragmented to form the peak at *m/z* 172. These results indicate that the major reaction intermediate between DCBQ and *t*-BuOOH was probably chloro-*t*-butylperoxyl-1,4-benzoquinone (CBQ-OO-*t*-Bu) (peak clusters at *m/z* 229), and the major reaction product between DCBQ and *t*-BuOOH was probably the ionic form of 2-chloro-5-hydroxy-1,4-benzoquinone (CBQ-OH) (peak clusters at *m/z* 157). This was confirmed by comparing with the synthesized authentic CBQ-OH, which showed the same ESI-MS profile and the same retention time in HPLC. Based on these data, we propose a novel mechanism for DCBQ-mediated *t*-BuOOH decomposition and formation of *t*-BuO$^{\bullet}$ (See scheme below): a nucleophilic attack of *t*-BuOOH on DCBQ, forming a chloro-*t*-butylperoxyl-1,4-benzoquinone intermediate, which decomposes homolytically to produce *t*-BuO$^{\bullet}$. This represents a novel mechanism of organic alkoxyl radical formation not requiring the involvement of redox-active transition metal ions.

Our observation that not only DCBQ, but also other chlorinated quinones can react with organic hydroperoxides to produce alkoxyl radicals in a metal-independent manner has interesting biological implications. For example, many widely used chlorinated aromatic compounds, such as hexachlorobenzene, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-

dichlorophenoxyacetic acid (2,4-D), and polychlorinated phenols, including the widely used wood preservative pentachlorophenol (PCP), can be metabolized in vivo to tetra-, di-, or mono-chlorinated quinones. Our data suggest that the chlorinated quinones may react with lipid hydroperoxides and exert toxic effects through enhanced production of alkoxyl radicals and, hence, increased lipid peroxidation. Additional work is needed to investigate whether these reactions occur and are relevant under physiological conditions or in vivo.



OP26

Protein oxidation and proteolysis in ageing

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Free radicals produced by cellular metabolism, xenobiotics, various forms of radiation, autooxidation, and chronic inflammation cause cumulative damage to cellular macromolecules, and appear to contribute to senescence/ageing and age-related (degenerative) disorders. Proteins are major targets for oxidative damage (in addition to DNA and lipids) and the accumulation of oxidized proteins has been reported in many ageing and disease models. In young and healthy individuals, moderately oxidized soluble cell proteins are selectively and rapidly degraded by the 20S Proteasome in the cell cytoplasm, nucleus, and endoplasmic reticulum. Inside mitochondria, the matrix proteinase called 'Lon' selectively degrades oxidized soluble proteins. The mechanism of selective proteolysis appears to depend upon oxidation-induced protein unfolding, with increasing surface hydrophobicity as (previously shielded) hydrophobic residues are exposed from the interior. The 20S Proteasome can preferentially bind to, and degrade such mildly oxidized, hydrophobic proteins without a need for ubiquitin targeting or ATP activation. The Lon protease appears to work similarly, except that its degradation of oxidized mitochondrial proteins is stimulated, up to five-fold, by ATP. Severely oxidized, aggregated, and cross-linked proteins, however, are poor substrates for degradation and actually inhibit the Proteasome and the Lon protease. During ageing, and in many age-related diseases/disorders, the Proteasome is progressively inhibited by binding to increasing levels of oxidized and cross-linked protein aggregates. Also during ageing, the levels of expression of the Proteasome and the Lon protease decrease significantly in certain cells and tissues. In young people, both the Lon protease and the Proteasome (as well as certain proteasome regulators) can be induced by exposure to mild oxidative stress. During ageing, however, the Proteasome and the Lon protease become significantly less responsive to stress-induction, thus limiting adaptive stress responses. Cellular ageing and inflammatory diseases probably include both an increase in the generation of reactive oxygen species as well as a decline in Proteasome and Lon protease activities and inducibility, resulting in the progressive accumulation of oxidatively damaged protein aggregates that eventually contribute to cellular dysfunction and senescence.

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OP27

Serum albumin glucoxidation impaired antioxidant defense in type 2 diabetes

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Epidemiological data consistently show that reduced levels of serum are associated with an increased mortality risk. Various biological properties evidenced by direct effects of the albumin molecule may explain its beneficial effects. Type 2 diabetes is also often associated with a reduced antioxidant defense evidenced by elevation of oxidation markers. In the present lecture, we aimed to report on antioxidant activities of native albumin as an inhibitor of copper-mediated LDL oxidation (OxLDL) and free radical-induced hemolysis. We found that ligand binding and free radical scavenging activities of albumin were altered by *in vitro* oxidation, and glycation as those observed in diabetes and also glucoxidation products (methylglyoxal). We also found that albumin modifications altered the structural characteristics of the albumin molecule as studied by tryptophan intrinsic fluorescence. These changes were related to impairment of the ligand-binding capacities of albumin. In a clinical study carried out in type 2 diabetic patients, we found that albuminemia was significantly reduced compared to controls. We also found that the antioxidant properties of albumin isolated from these patients were markedly impaired by comparison with albumin isolated from controls. Overall, these new data allow us to conclude that albumin and the modulation of its antioxidant activity play an important role in the free radical defense and further confirm the occurrence and metabolic impact of glucooxidant stress in type 2 diabetic patients.

OP28

Mechanisms underlying skeletal muscle weakness in the elderly

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Skeletal muscle has a unique ability to adapt rapidly to changes in the pattern of activity that it performs. Following unaccustomed and demanding contractile activity numerous structural and biochemical changes in muscle have been recognised to occur. During contractile activity skeletal muscle cells generate increased amounts of reactive oxygen species (ROS) through a number of different pathways and we have obtained data that indicate these contraction-induced ROS modulate at least some of the adaptive responses that occur in skeletal muscle following contractile activity. This process involves activation of redox-regulated transcription factors, such as AP-1, NFκB and HSF-1 and leads to increased expression of cytoprotective proteins that protect muscle cells against potential damage following subsequent rises in ROS activity. During ageing of skeletal muscle there is evidence that this ability to adapt to contraction-induced ROS fails with consequent increase in oxidative damage to skeletal muscle associated with the age-related loss of muscle mass and function. These changes are mimicked in young mice that show an accelerated ageing phenotype due to an increased activity of ROS resulting from knockout of the SOD1 gene, findings that support an involvement of excess superoxide activity in the ageing process in skeletal muscle.

OP29

Oxidation is a prerequisite for plaque cholesterol to exert up regulation of CD36 on monocytic cells and foam cell formation

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Within atherosclerotic lesions, the cholesterol molecule does not appear *per se* able to drive molecular mechanisms of progression unless inflammation is present. Of note, in fibrotic plaques containing phagocytes, the cholesterol oxidation product content (oxysterols) appeared closely proportional to the total cholesterol content. We thus investigated the effect of both unoxidized and oxidized cholesterol on monocytic cell differentiation and on foam-cell formation, as a possible key feature in atheroma progression. A biologically-representative mixture of oxysterols, but not the parent compound, was found to markedly up-regulate CD36 expression and synthesis in human U937 pro-monocytic cells. Up-regulation of this primary scavenger receptor by the oxysterol mixture was shown to involve the PKCδ, ERK1,2 and PPARγ pathways. Finally, cells overexpressing CD36 were shown to actively take up oxidized LDL particles, and eventually to be transformed into foam cells.

OP30

Nitrated-albumin-bound 3-NO_2-tyrosine denitration by g-tocopherol-free LDL - What can we infer from this novel LDL activity?

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Human serum albumin (HSA) is partly associated to LDL to form a LDL-albumin complex (LAC) recently found to be natively poorly nitrated (Carbonneau et al, Free Rad Res, 2002, 36 :127 ; Torres-Rasgado et al, Free Rad Res, 2007, 41, in press). Added HSA decreased the LAC-tyrosine nitration elicited in mild, physiological conditions. Is LAC able to denitrate HSA-bound 3NT ? Nitrated HSA (3NT/total-Tyr = 0.1) was incubated in the presence of 2 types of LAC (HSA-rich- or HSA-low-LDL, molar ratio 15 :1 and 1.2 :1, respectively), Ca^{2+} and an anti-proteolytic mixture (APM). After acetonitrile precipitation and centrifugation, supernatant (SN) was removed, protein pellet (protein fraction PF) was washed and resuspended for HCl-proteolysis in the presence of 3-NO_2-[d_3]-tyrosine as internal standard. Amino acids were derivatized and analyzed using a GC-MS procedure. Quantification was carried out from a regression line obtained by plotting the peak area ratio of non-*d*- to d_3-3NT selected fragments for each incubation condition. An important loss of 3NT was found in PF only in the presence of Ca^{2+}. It was higher when nitrated HSA was incubated with HSA-low LDL. In the absence of APM, the loss was more pronounced but no difference occurred due to the presence/absence of Ca^{2+} or types of LAC. Loss was considerably impaired by an inhibitor (D-penicilamine) or a substrate in excess (phenylacetate) of PON1. After g-tocopherol LDL-overloading, no loss took place. In any condition, loss assessing was corroborated by nitrate fluorometric assays on SN. HSA denitration by a paraoxonase-like activity associated to g-tocopherol-free LDL (and to HDL, not reported) we presently found leads to speculate on the fate of the nitrated-HSA released NO_3^- known to be be converted into $NO^{\bullet}$ by xanthine reductase.

OP31

The role of oxidative stress in hemolytic anemias and the antioxidant effect of Fermented Papaya Preparation *in vivo and in vitro*

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The oxidative status modulates various normal physiological cell functions, such as signal transduction. On the other hand, oxidative stress is cytotoxic - leading to apoptosis and organ damage and contributes to the pathogenesis of many diseases. We studied the role of oxidative stress in hemolytic anemias: hemoglobinopathies (thalassemia and sickle cell anemia); paroxysmal nocturnal hemoglobinuria; G6PD deficiency and the myelodysplastic syndrome. For these studies we adapted flow cytometry techniques measuring the various parameters of oxidative stress in blood cells. The results show that in all these diseases, red blood cells (RBC), platelets and polymorphonuclear (PMN) leukocytes were under oxidative stress: Increased generation of Reactive Oxygen Species (ROS), membrane lipid peroxidation and externalization of phosphatidylserine moieties, concomitant with decrease in the content of reduced glutathione (GSH) were found at basal state and following stimulation by oxidants. These findings may explain major symptoms in these diseases: Oxidative stress in RBC is responsible for anemia (due to ineffective erythropoiesis of erythroid precursors in the bone marrow and short survival time of the mature RBC in the peripheral blood). Oxidative stressed platelets have increased tendency to undergo activation and aggregation and may account for the high incidence of thromboembolic complications, and PMN under stress may have ineffective bacteriocidal activity, resulting in recurrent infections. Fermented papaya preparation (FPP) has been previously shown to reduce oxidative stress. We tested its antioxidant effects on RBC, platelets and PMN of ? -thalassemic mice and patients. *In vitro* experiments showed that treatment with FPP reduced the aforementioned oxidative stress parameters in thalassemic RBC and increased their survival *in vitro* by preventing hemolysis and by reducing their susceptibility to undergo phagocytosis by macrophages.

The effect on thalassemic mice was studied by adding 10 mg /ml FPP to their drinking water. The results demonstrated that treatment with FPP significantly reduced all the tested parameters of oxidative stress. Based on these results we treated a group of 9 patients with ? -thalassemia for 12 weeks. In all patients ROS was markedly lower associated with reciprocal increase in GSH in RBC, platelets and PMN. These results suggest that FPP can decrease oxidative stress in blood cells both *in vitro* and *in vivo*. The effects observed *in vivo* suggest that FPP may have important clinical efficacy in thalassemia as well as in other hematological pathologies.

P1

Non-invasive biomarkers of oxidative stress (volatile aldehydes) in preterm infants and in children with cystic fibrosis (CF)

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Background **:**
Oxidative stress is an important outcome factor in many pediatric diseases as bronchopulmonary dysplasia or CF.
Objectives : To develop non-invasive measurements of volatile aldehydes as early indicators of oxidative stress in preterm infants (PT) receiving oxygen therapy or in children with cystic fibrosis (CF).
Méthods : Volatile aldehydes (MDA, propanal, hexanal, pentanal) were measured in the urine or in exhaled breath condensates (EBC) by gas chromatography-electron-capture detection. We studied 88 very low birth weight infants (< 31 wks GA). Urine collections were performed on day 1, 2, 3, 4, 5, 6, 7, 14, 21, 28 post natal age and after normalization of oxygen needs in PT. In 5 CF children, measurements were made in both the urine and BEC collected by Ecoscreen and compared to 3 healthy adults. Ethic comity consent was obtained.
Results : The coefficient of variation of the aldehyde analysis was 5,1 % in the urine of PT. For definitive analysis, 100 PT must be included (89 at present). In children with CF, preliminary results shown a higher level of MDA (0,59 ± 0,17) in EBC compared to healthy adults (0,06 ± 0,17; $p< 0,05$). Urinary MDA levels in CF children (1,69 ± 0,61) was greater than in EBC (0,59 ± 0,17, $p< 0,05$).
Comments and conclusion : These volatile aldehydes appear to be useful as precise and non-invasive biomarkers of oxidative stress in both PT at risk of developing bronchopulmonary dysplasia and children with CF.
Aknoledgment to **Memorial A. de Rothschild**.

P2

An electroanalytical method using electrogenerated superoxide anion radical for the *in vitro* determination of antioxidant capacity of flavonoids and seaweed extracts.

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Various *in vitro* and *in vivo* methods have been developped to measure the antioxidant capacity of molecules using spectrophotometric, fluorescence or EPR techniques. Electrochemical methods are also useful to antioxidant research, one of their interests is that these techniques allows the generation of radicals and the measure of their reaction towards antioxidant species.

This work reports the validation of an electroanalytical method to measure the *in vitro* antioxidant capacity of phenolic compounds. The methodology is based on the study of the interaction of the substrate with superoxide anion radical $O_2^{\cdot -}$, well-known as a reactive oxygen species (ROS). A voltammetric technique is used to directly generate the radical on an electrode by reduction of the oxygen in aprotic media and to measure the oxidation current decay of the radical in presence of increasing concentrations of substrate. A linear dependence $O_2^{\cdot -}$ oxidation current *vs.* substrate concentration is observed and allows to determine IC50 values, defined as the substrate concentration needed to reduce 50% of $O_2^{\cdot -}$.

Statistical analysis of the linearity and the precision (intra and interlaboratory) of the method was performed on a flavonoid series and standard antioxidants (Trolox, ascorbic acid, phloroglucinol). The validated method was applied to the determination of the antioxidant capacity of polyphenol seaweed extracts.

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P3

Field test of a DNA micro-array for the diagnosis of oxidative stress

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We have developed a DNA micro-array containing 200 genes involved in the oxidative stress metabolism and in inflammation. The array was used to study the expression of these genes in a cohort of hundred healthy donors in parallel with various biochemical markers. The aim of the study was to determine if the array was able to distinguish genes expression profiles specific to physiologic and environmental factors that are known to influence the oxidative stress.
We observed significant correlations between various groups of genes as well as between certain genes and certain biochemical markers. Many genes were differentially expressed in relation with the BMI, the use of oral contraception or the physical activity. For many of these genes, the differences of expression that we observed between the groups were statistically significant.
The biochemical tools allow the assessment of oxidative and anti-oxidative substances in the blood, the urines or the tissues. The biochemical balance gives a snapshot image of the state of the oxidative stress of the person and reflects well the dietary contributions or the supplementation in anti-oxidants. It brings however only few information about the physiopathology mechanisms and does not take into account genetic predispositions. The measurement of the expression of the genes involved in the oxidative metabolism brings a better knowledge of the functions of the genes and of the metabolic pathways involved in the oxidative stress. To assess the power of the micro-array for the diagnosis of oxidative stress in different clinical situations, we are now conducting clinical trials in different fields including diabetes, obesity, coronary bypass, liver graft and intensive physical activity.

P4

The effect of a dietary supplementation with a-tocopherol and folic acid on 8-oxodG, a biomarker of oxidative stress, in leukocytes and urin of humans

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In the present study we measured the level of 8-oxo-2'-deoxyguanosin (8-oxodG) in urine and in leukocyte DNA from 15 healthy male subjects. The purpose of the study was to evaluate the oxidative stress including factors that may influence oxidative DNA damage, such as smoking or the vitamins a-tocopherol and folic acid. The urinary level of 8-oxodG is a marker of the average total oxidative stress to DNA of all body cells. The concentration of 8-oxodG in the leukocytes reflect a balance between oxidative DNA damage and repair of the damage in the cells.
Within the scope of the intervention study the 15 subjects received daily 270 mg a-tocopherol and 600 µg folic acid over 4 weeks. For the determination of 8-oxodG and 2'-deoxyguanosine (dG) in the leukocyte DNA and urine a HPLC-ECD and HPLC-DAD method was used. The plasma concentration of tocopherols, 5-methyltetrahydrofolate (5-MTHF) as well as homocysteine was analyzed by means of HPLC and DAD or fluorescence detector.
The Supplementation with a-tocopherol and folic acid caused a significant decrease of the 8-oxodG/10^5dG concentrations in the leukocyte. For the whole group the 8-oxodG/10^5dG concentrations was significantly ($p < 0.01$) decreased by about 78% after 4-week supplementation. The reduction of the 8-oxodG/10^5dG concentrations was higher in smokers (87%) than in non-smokers (72%). No significant difference between smokers and non-smokers could be determined in leukocytes, neither at the study beginning (0.67 8-oxodG/10^5dG and 0.61 8-oxodG/10^5dG) nor at the study end (0.19 8-oxodG/10^5dG or 0.17 8-oxodG/10^5dG). The concentration of 5-MTHF in the plasma rose significantly (about 39%, $p < 0.01$). At the same time the homocysteine concentration was significantly lowered in the plasma (by about 19%, $p < 0.01$). The plasma concentration of a-tocopherol was increased by about 60%.

Parameters	study begin	study end	*p*
8-oxodG/10^5dG all	0.64	0.18	<0.01
- smoker	0.67	0.19	<0.01
- non-smokers	0.61	0.17	<0.01
5-MTHF [µg/L]	7.47	10.35	<0.01
homocysteine [µmol/L]	10.42	8.49	<0.01
a-tocopherol [µg/mL]	12.71	20.32	<0.05

Furthermore a significant correlation between a-tocopherol concentration in plasma and 8-oxodG/10^5dG ($r = -0.36$) in leukocyte DNA could be proven ($p < 0.05$). No significant correlation was found between the urinary concentration of 8-oxodG and the concentration of 8-oxodG in leukocyte DNA

P5

Involvement of reactive oxygen species in surface erosion of mineral fibres in human macrophages: the role of *NCF-1* gene.

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Occupational pulmonary diseases initiated by inhalation of mineral fibrous particulates result from a chronic inflammatory process in which alveolar macrophages play a crucial role. Actually, alveolar macrophages are responsible for clearance of particles that reach the deep lung. Because of their fibrous morphology, man made mineral fibres (MMMF) are extensively submitted to European codified protocols using rodents, in order to study their toxic potential and biopersistence in lung. Thus, insulation wool fibres removed from lung rodents 30 days after their inhalation, displayed surface erosions as holes, probably due to their degradation by pulmonary macrophages.
Using human monocytes (U-937) activated by *Escherichia coli* extracts, we were able to reproduce such fibre degradation observed in rodents. We evidenced a strong inverse relationship between fibre biopersistence in deep lung and their erosion using the *in vitro* test we developed. Moreover, fibre phagocytosis and sustained degradation modified gene expression profile in macrophage. We performed a molecular analysis of gene expression in macrophage using a 20-k DNA chips.
Transcriptome analysis highlighted the influence of oxidative stress in activated macrophage in presence of MMMF. One of the most representative over-expressed genes was *NCF1* gene that encodes the p47 subunit of the NADPH oxidase complex. Over-expression of this gene was confirmed by a qRT PCR experiment. NADPH oxidase complex, during phagocytosis, catalyses generation of superoxide that is a biomarker of oxidative stress. This finding leads us to hypothesise an active role of ROS in fibre degradation and in their biopersistence. Thus, we designed an experiment in which fibres were incubated in a hydrogen peroxide solution. They showed similar surface erosions to those displayed *in vivo*. Therefore, we propose a molecular mechanism for *in vivo* fibre degradation through *NCF1* gene activation in macrophages. Indeed, using this *in vitro* acellular test, we were able to obtain results similar to those obtained *in vivo*. Both cellular and acellular *in vitro* tests mimic physiological function of lung particle degradation and dissolution.
The endpoint we used, namely oxidative stress induction in macrophages through *NCF1* gene activation, may be applied in oxidative stress studies using Langerhans cells or keratinocytes. These studies may be of biological interest especially with the increased use of nanoparticles in skin care formulations.

P6

Effect of different contraception methods on the oxidative stress status in women aged 40-48 years from the ELAN study in the Province of Liège, Belgium. (Accepted for publication in Human Reproduction, 2007)

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Background:
Oxidative stress may contribute to the development of several disorders including cardiovascular disease and cancer. Among conditions known to influence oxidative stress, the use of oral contraception (OC) in women has been a matter of ongoing discussion.

Methods :
A total of 897 eligible and healthy volunteers were recruited among the practice of 50 general practitioners participating to the ELAN study (Etude Liégeoise sur les ANtioxydants). A subsample consisting of 209 women aged between 40-48 years was studied for a comprehensive oxidative stress profile, including the analysis of antioxidants, trace elements and three markers of oxidative damage to lipids. Forty - nine (23%) of these women were oral contraception users (OCU), 119 (57%) non-contraception users (NCU) and 41 (20%) intrauterine (hormonal and copper) devices users (IUD).

Results
After adjustment for smoking systolic and diastolic blood pressures and body mass index (BMI) (or waist circumference), a marked and significant increase in lipid peroxides was observed among OCU women when compared to NCU and intrauterine IUD subjects. A cut-off value of 628-660 µM in lipid peroxides allowed to discern OCU from the two other groups. In contrast, no difference was observed in the plasma concentration of both oxidized LDL and their related antibodies. The increased level in lipid peroxides was strongly related to higher concentrations in copper ($r = 0.84$; $p < 0.0001$, cut-off value 1.2 mg/L). When compared to NCU and IUD, plasma antioxidant defences were significantly altered in OCU women as shown by lower levels in β-carotene (decrease of 39 %, $p < 0.01$) and γ – tocopherol (decrease of 22 %, $p < 0.01$) and higher concentrations in selenium (increase of 11.8 %, $p < 0.01$). The blood concentration in vitamin C, α-tocopherol and zinc was unaffected by OC use.

Conclusions
The intake of oral contraception significantly exacerbates the lipid peroxidation in women aged 40-48 years. This is clearly related to an increase in plasma copper concentration. This may represent a potential cardiovascular risk factor for these women.

P7

Intake of fruits and vegetables in men and women aged 40 – 60 years from the ELAN study* in the Province of Liège, Belgium.

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Background

The regular intake of fruit and vegetable (5 servings per day) known for their high content in antioxidant is thought to be associated with a lower incidence in the development of cardiovascular diseases and cancer. In the province of Liège, Belgium, we performed a large study for evaluating the intake of such nutrients and their impact on plasma antioxidant concentrations that are vitamin C and β – carotene.

Methods

A total of 868 eligible healthy volunteers (339 men and 529 women aged 40 – 60 years) were recruited among the patients of 50 general practioners participating to the ELAN study (Etude Liégeoise sur les ANtioxydants). This study was conducted from March through July 2006 in the Province of Liège, Belgium. A home – made food frequency to assess fruit and vegetable intake during the month preceding the visit to their physician's office was completed by all participants. Individual vitamin C and β – carotene intake was calculated at the Department of Dietetics according to German database (Souci, Fachmann, Kraut, 2000). The day of the visit, a blood sample was drawn for the determination of vitamin C and β –carotene according to the good laboratory practice.

Results

Intake of fruit

Among women the following pattern was observed: none: 11.53%, 1 and sometimes 2 servings per day: 57.66%; 2 to 3 servings per day: 23.25%; > 3 servings per day: 7.56%. For men, a significant increase ($p < 0.0001$) was noted in the first group: 21.83% as well as a significant decrease ($p < 0.0001$) in the third group: 14.75%. In both groups, smoking habits leaded to a less intake of fruit ($p < 0.0001$) when compared to non – smokers: none: 24.43 vs 12.56%; 2 to 3 servings per day: 12.56 vs 22.95%; > 3 servings per day: 4.07 vs 9.15%. During all the time of the study, the 5 fruits the most frequently consumed by both men and women were as follows: apple (75.25%), banana (56.45%), orange (43.9%), peer (men: 25.5%; women: 19.1%, $p < 0.0001$) and kiwi (men: 12.6 %; women: 17.1%). On May, the intake of strawberry (a Belgian speciality from Wepion) and grape respectively reached 23.35% and 17.8%. Except for mandarin (12%), the intake of all other fruits was largely below 5%.

Intake of vegetables

Among 12 vegetables cited by the participants, the food – frequency questionnaire did not revealed significant difference in usual vegetable intake between men and women: none: 26.8%, low intake: 36.5%; regular intake: 36.65%. No clear effect of the smoking habits was noted. During all the time of the study, the 5 vegetables the most frequently consumed by both men and women were as follows: lettuce (67.35%), tomato (58.15%), carrot (34.25%), cabbage including broccoli and Brussels sprouts (32.3%) and bean (27%).

Effect of fruit and vegetable on antioxidant intake and their blood concentration

The dietary intake in both vitamin C and β – carotene of the ELAN subjects significantly differed according to the sex. In men, we observed a daily intake in vitamin C of 118 +/-71 mg (75.1 +/-58.7 mg from fruit and 43.26 +/- 25.72 mg from vegetables). In women, a significant higher value ($p < 0.0001$) was found: 142 +/- 58 mg (95.5 +/- 58.7 mg from fruit and 47.3 +/- 27.8 mg from vegetables). With respect to β - carotene, the daily intake for men was: 3.98 +/- 2.53 mg (1.23 +/- 1.91 mg from fruit and 2.81 +/- 1.62 mg from vegetable). Those of women were significantly higher ($p < 0.0001$): 4.87 +/- 3.07 mg (1.8 +/- 2.17 from fruit and 3.05 +/- 1.74 from vegetable).

After adjustment for sex and smoking habits, statistical analysis revealed that much higher was the consumption of fruits and much higher were the plasma concentrations in both vitamin C and β – carotene. Among fruit, orange intake clearly appeared to have the more significant impact on both plasma vitamin C ($p < 0.02$) and β – carotene ($p < 0.05$) level. With respect to vegetable, a higher intake in carrots ($p < 0.005$) and Brussels sprouts ($p < 0.05$) resulted in a significant higher plasma concentration in β - carotene. However, this effect seemed to disappear after adjustment for sex. From an unvaried point of view, no particular vegetable had a significant impact on the vitamin C level, except for the regular intake of Brussels sprouts which surprisingly tended to decrease the vitamin C concentration.

Conclusions

As found in other studies, we confirm on the basis of a simple food frequency questionnaire that women tend to consume more fruit and vegetable than men. This is, however, strictly limited to apple, orange, banana among fruit and lettuce, tomato, carrot and cabbage (including broccoli and Brussels sprouts) among vegetable. Even after adjustment for sex, it is evident that a higher intake in fruit results in an increased level in both vitamin C and β – carotene. Orange seems to be the major fruit influencing both parameters. In contrast, no particular vegetable reveals such an effect, except for carrots and Brussels sprouts on the plasma level β – carotene. As general conclusions, the ELAN study (men and women aged 40 - 60 years) highlights two points: 1° the large majority of both men and women aged 40 – 60 years is far to apply the 5 – a – day concept; 2° there is no diversity (and colour) in the intake of fruit and vegetable.

P8

Nucleotides reduces DNA Damage Induced by Ionizing Radiation Exposed Lymphocytes *In Vitro*

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The objective of present study was to determine the effect of nucleotides in reducing DNA damage induced by ionizing radiation on human lymphocytes *in vitro*. Human peripheral blood lymphocytes were irradiated with 3 Gy Gamma rays and cultured in media 199 with and without nucleotides supplementation. DNA-damage was assessed in term of DNA fragmentation, apoptosis and frequency of micronuclei. Results showed that nucleotide supplementation significantly decreased the increase in DNA fragmentation, apoptosis and the frequency of micronuclei induced by ionizing radiation in lymphocytes in vitro. The results obtained suggest that nucleotides supplementation have the potency to reduce the extent of DNA damage induced by ionizing radiation.

Non Small Cell Lung Cancer Molecular Biomarkers in Blood

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Several studies have reported significant correlations between individual telomerase activity and apoptosis in malignant tumours including lung cancer. Such studies were carried out on tissue biopsies from the malignant tissue. The aim of this present study was to find molecular biomarkers in blood that can be used for early detection of non small cell lung cancer. Telomerase activity and Bcl2 anti-apoptotic protein in circulating lymphocytes, plasma nitric oxide, epidermal growth factor and epidermal growth factor receptor were measured in the blood of 25 non squamous cell carcinoma patients and in 20 normal age and socio-economic matching controls. Results revealed significant increase in telomerase activity (53 ± 8.2 vs. 19.5 ± 4.8, $p<0.0001$) between cancer patients and normal controls, significant lower levels of Bcl2 (7.2 ± 1.5 vs. 10.4 ± 1.4 µg/ml, $t = 7.3$, $p < 0.0000001$) among cancer patients compared to normal controls. However there were significantly higher levels of epidermal growth factors (6.2 ± 2.05 pg/mg vs. 0.2 ± 0.01 pg/ml), epidermal growth factor receptor EGFR (134 ± 4.7 vs. 102 ± 2.2 fmol/ml) and plasma nitrate/nitrite ratio (18.8 ± 4.7 vs. 12.5 ± 5 µg/ml, $t = 5.25$, $p < 0.0001$) in lung cancer patients compared to controls. This study reveals that Bcl2 levels in circulating peripheral blood are weak biomarkers for lung cancer, while increase in plasma telomerase activity and NO levels and EGF can be used as simple indicators for cancer prognosis.

P9

Induction of DNA repair in the brain of vitamin C deficient neonatal guinea pigs

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Induction of DNA repair has not previously been shown in vivo and in vitro experiments have so far suggested that DNA repair is not inducible by increased oxidative stress. Neonates are particularly susceptible to oxidative stress due to their limited reserves of micronutrients and their rapid growth. In the present study, we examined the effect of vitamin C deficiency on markers of oxidative stress and DNA repair in brain and liver of neonatal guinea pigs. We were particularly interested in the brain, since it is highly susceptible to oxidative damage and known to accumulate high concentrations of ascorbate, the reduced and antioxidant active form of vitamin C. Vitamin C deficiency caused rapid and significant depletion of ascorbate ($P<0.001$), tocopherols ($P<0.001$), and glutathione ($P<0.001$) and a decrease in SOD activity ($P<0.005$) in the liver, while protein oxidation was significantly increased ($P<0.05$). No changes in lipid oxidation, oxidatively damaged DNA or DNA repair were observed in this tissue. In the brain, the pattern was markedly different. Of the measured antioxidants, only ascorbate was significantly depleted ($P<0.001$), but in contrast to the liver, ascorbate oxidation ($P<0.05$), lipid oxidation ($P<0.001$), DNA oxidation ($P=0.13$) and DNA incision repair ($P<0.05$) were all increased, while protein oxidation decreased ($P<0.005$). Our results show that DNA repair is induced by vitamin C deficiency in the brains of neonatal guinea pigs. However, the selective preservation of brain ascorbate and induction of DNA repair is not sufficient to prevent oxidative damage. Vitamin C deficiency may therefore be particularly adverse during the neonatal period.

P10

Beta-carotene and arachidonic acid – induced changes in human endothelial cells and its progenitors .

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DNA methylation is a mechanism regulating gene expression . Beta-carotene (BC), potent pro-vitamin A/retinoic acid source in human, was shown to have pro-chemotactic activity and stimulate expression of pro-angiogenic genes in endothelium. Angiogenesis, , is an important mechanism in tumour malignancyFatty acids stimulate BC uptake. The arachidonic acid (AA) metabolites were shown the procancerogenic activity. This study was undertaken to define the possible changes in DNA methylation in endothelial cells and its progenitors after incubation with BC and AA.

Human umbilical vein endothelial cells (HUVEC) and isolated from cord blood endothelial progenitors (EPC) were incubated with BC (1-10μM) and 3μM arachidonic acid (AA) for 24 hours. The CpG island methylation was quantified using the Combined Bisulphite Restriction Analysis (COBRA) method (HotStarTaq Master Mix Kit, Qiagen) and the PCR products were digested by restriction enzymes (NewEngland BioLabs). Global DNA methylation was analysed with cytosine extension assay using methyl-sensitive restriction enzyme HpaII (New England Biolabs), which allows [^{3}H]dCTP to be incorporated into the DNA strands..

The global DNA methylation analysis pointed to the tendency to down-regulation of DNA methylation in HUVEC and EPC, after incubation with AA ($p=0.919$) or BC ($p=0.227$). Of the 18 investigated genes connected with the endothelial cell proangiogenic activity, DNA methylation was regulated in the promoter regions of : *integrinb3, connexin 43, CXCR4, KDR, MMP-2, laminin, Notch4* and *VCAM1* genes .

Conclusion: The CpG island methylation might be an important mechanism of changes in the expression of pro-angiogenic genes after stimulation with beta-carotene and arachidonic acid. The presented results are from a pilot study and need further observation.

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P11

The chemotactic activity of beta-carotene in endothelial cell progenitors and HUVEC. The microarray analysis.

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Endothelial cells and its progenitors play an important role in angiogenesis, essential for organogenesis, tissue remodeling but also for inflammatory response, carcinogenesis in all periods of our life. In our study we concentrated on the direct effect of beta-carotene (BC) on human umbilical cord originated endothelial progenitors (EPC) and human umbilical vein endothelial cells (HUVEC).
Methods: BC uptake was measured by HPLC method. The effect on cell proliferation was measured by BrdU incorporation. Chemotaxis was performed in the Boyden's chamber The influence on tubular-like structure formation was investigated by the 3D assay in matrigel in vitro as well as in vivo model. Changes of gene expression were analyzed using microarray hybridization method. The quantitative gene expression was estimated using the real-time PCR method.

Results: We have demonstrated that BC-in the physiological range of concentrations, found in human blood, is a potent activator of chemotaxis of endothelial cells. The microarray data analysis revealed that the genes involved in cell/cell; cell /matrix adhesion; matrix reorganization, and activation of chemotaxis were the most affected by BC genes in HUVEC and EPC. These results were also confirmed in in vivo angiogenesis model.
Conclusion: BC in the physiological concentration range stimulates early steps of angiogenic activity of endothelial cells by activation of cellular migration as well as matrix reorganization and decrease of cell adhesion.

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P12

The modality of combined supplementation with vitamin E and C and the predictive value of hsCRP, ischemia modified albumin (IMA) levels as the markers of oxidative stress in patients at cardiovascular risk.

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Background: Plasma hsCRP and ischemia modified albumin (IMA) are recently introducea as the new cardiovascular risk markers. **The aim** of study was to assess the relation between the modality of vitamin intake with plasma markers of oxidant stress and the effects of short-term dietary supplementation with vitamin E and C on hsCRP, IMA and plasma oxidative stress/antioxidant capacity parameters. **Patients and method**s. Combined supplementary vitamin E and vitamin C (2 x 100mg and 2 x 200mg daily, respectively) was administered to 108 men on an empty stomach or during dinner, for 14 days in the cross-over study. We selected control, healthy men (n=20), cardiovascular event-free obese subjects (n=38) and peripheral vascular disease (PVD) patients after surgical revascularization (n=44). Plasma hsCRP, IMA, parameters of oxidative stress: TBARS, LOOH, LDL oxidative susceptibility, and antioxidant potency: FRAP, thiol/albumin ratio, vitamin E, C, redox compensation index were assessed. **Results:** Food intake increases Vitamin E and C absorption . This results in the decrease of plasma oxidative stress parameters . The PVD angioplasty improves blood perfusion, but did not reduce the oxidative stress, markedly increased compared to control and patients with obesity. VitE+C supplementation significantly reduced plasma oxidative stress and improved plasma antioxidant potential more efficiently in subjects representing the highest quartile of hs-CRP and IMA levels. **Conclusion**: Meals increase the bioavailability of antioxidant vitamins and improves their free-radical-scavenging activity. Plasma hsCRP and IMA serve as potential marker of oxidative stress and may be useful for selecting patients who are candidates for supplementation with antioxidant vitamins

P13

Inhibition of lupus-associated oxidative stress by Conjugated Linoleic Acid paralleled by phase 2 enzymes activation.

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Cis_9, $trans_{11}$ and $trans_{10,}$ cis_{12} Conjugated Linoleic Acid (CLA) isomers, predominantly present in foods from ruminant animals, exhibited anti-carcinogenic and anti-inflammatory properties. Oxidative stress is known to be implicated in autoimmunity and recent evidence supports an antioxidant basis for the anti-inflammatory activity of CLA in Systemic Lupus Erythematosus (SLE), as evidenced by the association of enhanced expression of gamma-Glutamylcysteine Ligase (γGCL) with the amelioration of autoimmune signs in a murine model of human SLE (MRL/*lpr* mice). As this enzyme belongs to a group of enzymes (phase 2), crucial for protecting cell against oxidative stress, the aim of the present study was to examine CLA ability to modulate redox status and phase 2 enzymes activity, namely NAD(P)H:quinine oxidoreductase (NQO1), Glutathione *S*-transferase (GST) and γGCL, in MRL/*lpr* mice.
Age-dependent oxidative stress in murine SLE was first confirmed by decreased GSH concentration and enhanced levels of oxidative stress markers (protein carbonyls, nitrosylated protein and anti-dsDNA IgGs) in diseased mice (20-22 wks old) as compared with pre-diseased animals. Next, enhanced GSH concentration in serum of diseased MRL/lpr mice, administered for two weeks with CLA (30 mg/day), as compared with animals receiving olive oil (control), and its negative correlation with oxidative stress markers content demonstrated the beneficial effect of CLA on animal redox status. Finally, phase 2 enzyme involvement was indicated by the enhanced NQO1, GST and γGCL activities in spleen and liver of CLA-treated animals as compared with controls. Presented results provides the first evidence of CLA ability to activate phase 2 enzymes and to decrease SLE-associated oxidative stress.

P14

Impact of Methylene Blue pathogen inactivation on plasma proteins

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Transfusion safety is a crucial parameter for patients After plasma collection, it is necessary to include a pathogen inactivation step in order to remove viruses. Among the different techniques that exist, Methylene Blue (MB) pathogen inactivation is becoming a standard method in blood centers worldwide. MB, in presence of oxygen and light, produces free radicals that will involve the modification of guanines in nucleic acid and thus provoke the break of the nucleic acid single strand. This enables the removal of virus fitted with a nucleic envelope.

However the impact on plasma proteins still needs to be assessed. We report here on our comparative study of MB inactivated plasma by 2D gel electrophoresis and nanoLC-nanoESI-FTICR mass spectrometry in order to assess the impact of such a treatment.

Fresh frozen and MB transfusion grade plasma provided by the *Etablissement Français du Sang* were separated by two-dimensional gel electrophoresis in order to map the plasma proteins; fresh frozen plasma being considered as the closest sample from native plasma. We noticed a tremendous change in protein profiles with the appearance of stripes suggesting oxidation modifications. The analysis by nanoLC-nanoESI-FTICR MS will enable us to identify the modifications induced by MB treatment and assess its impact particularly on fibrinogen.

P15

Sulforaphane as a new cardioprotective agent against oxidative damage

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The increasing recognition of the role of oxidative stress in the pathophysiology of cardiovascular diseases has led to extensive investigation on the protection against oxidative cardiac injury by exogenous antioxidants. Several naturally occurring compounds are known to present detoxicating properties in different mammalian cells, mostly by their ability to induce Phase II enzymes, but data on the ability of functional components of food to increase endogenous antioxidant defences are lacking. As an important consequence of Phase II enzyme induction is the enhancement of cellular antioxidant capacity, the up-regulation of endogenous antioxidant systems may represent a promising strategy for protecting cells against oxidative damage.
Using cultured rat cardiomyocytes we have characterized the time-dependent induction of cellular antioxidants and Phase II enzymes by sulforaphane (SF), a potent natural chemopreventive compound present in substantial quantities in the human diet (primarily originating from the ingestion of Cruciferous vegetables).
Incubation of cardiomyocytes with SF resulted in a marked increase of glutathione reductase, glutathione-S-transferase, quinone reductase 1 activity and expression and of intracellular GSH (the most prominent intracellular thiol in the heart) levels. SF pretreatment caused a decreased intracellular accumulation of ROS and an increased cell viability in comparison to cells exposed to oxidants. Our results demonstrate that SF influences the intracellular redox environment up-regulating antioxidant cellular defences, thus acting as an indirect antioxidant leading to cardioprotection against oxidative damage.
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P16

Whey protein inhibits food intake and tends to ameliorate oxidative equilibrium in Zucker Rats

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Whey proteins represent a heterogeneous group of proteins obtained from milk after casein precipitation. Whey protein supplementation in the diet may represent a possible adjuvant therapy in oxidative stress-correlated pathologies. Moreover, whey proteins seem to inhibit food intake. This work evaluates the effects of whey proteins in comparison with casein, as sources of alimentary proteins, on food intake, weight growth and some indexes of oxidative equilibrium in Zucker Rats, genetically prone to obesity. Treatment with whey protein resulted in significant decrease of food intake; the weight growth was analogously reduced. Whey proteins induced a slight increase of total glutathione both in the liver and in the blood. TBA-test did not revealed any significant difference, while significant decrease of plasmatic 4-hydroxynonenal was detected in the group receiving whey proteins. The effect of whey proteins on food intake in Zucker Rats is particularly noticeable, since the genetic nature of their predisposition to obesity; the possible parallel amelioration of oxidative balance may constitute a further advantage of whey proteins, since oxidative stress is believed to contribute to the complications of aging, obesity and metabolic syndrome.
This study has been supported by Advanced Food Research/AFR (Bollate, Milano, Italy)

P17

The effect of vitamin E-derivative on the biochemical properties of cardiomyocytes' plasma membranes under oxidative stress

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The purpose is to study the influence of vitamin E-derivative with shortened to C6 side chain (C6) (metabolite of a-tocopherol) on biochemical and functional state of cardiomyocytes' plasma membranes (CPM) of cholesterol-fed rabbits (hypercholesterolaemia, HC).
Levels of cholesterol and free fatty acids were increased and phospholipids level was decreased in CPM under HC. Proposed mechanism of these changes is intensification of free-radical oxidation (FRO), accompanied by significant accumulation of diene conjugates, malondialdehyde, and intensification of spontaneous and induced chemiluminescence of CPM. At the same time Na+, K+-ATPase activity and speed of Na+/Ca2+-exchange of CPM under HC were decreased. HC was not accompanied by changes of catalase and superoxide dismutase activity. But the glutathionreductase activity was decreased.
The normalization of lipid structure, decrease of intensity of FRO, prevention of Na+, K+-ATPase activity and Na+/Ca2+-exchange, increase of glutathionreductase activity were found under conditions of supplementation of C6 with therapeutic and prophylactic purpose per os at the concentration of 5 mg/kg for 30 days to animals with HC. C6 provides protection against the oxidation stress. We suggest that modification of molecule's side chain modulates high effectiveness of C6.
These results prove antioxidant properties and effective membrane-stabilizing activity of C6 and provide ground for using of C6 as an adaptogene for prophylaxis and treatment of cardiovascular pathologies and obtaining the new generation of vitamin E drugs.

P18

Effect of nonspecific stress factors on the respiration and thermotolerance of mammalian cells

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The antihypoxic effect of hypoxen (sodium salt of poly(2,5-dihydroxyphenylene)-4-thiosulfonic acid) is considered proven, but the mechanism of its action at the cellular level still remains elusive. In the present work we studied the effect of hypoxen on the functional state of 3T3 and BHK cells under the conditions of hypoxia and hyperthermia. In particular, we have measured cellular respiration and found that in hypoxen-treated cells the oxygen consumption decreases by 30% of control, which may indicate a more economic utilization of oxygen by cells. At the same time, there was no notable effect on the parameters of phosphorylation.

It was also examined thermotolerance of cell cultures. The G0-synchronized 3T3 cells were cultivated at 37°C, the time of cultivation being varied from 1 to 10 h. This was followed by a sharp jump in temperature to 43°C. In the control experiments, this temperature was maintained for 15 h. In other regimes, temperature was returned to 37°C for 2.5 h (after a 30 min incubation at 43°C) and then raised again to 43°C, eventually remaining there for 15 h. In the latter case, the number of cells survived by the end of experiment increased dramatically – all over the examined region of cell cycle except the period that precedes S-phase by 3-5 h.

P19

The methaboloc activator of mitochondria ATP-dependent potassium channel in oxidative stress

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We found earlier that UDP is a metabolic activator of the mitochondria ATP-dependent potassium channel (mitoK_{ATP}) (Mironova et al.J.Biol.Chem.2004, 279:32562). Here we present data showing that this activator decreases the myocardial damage seen after oxygen stress. It was shown that precursors of UDP (uridine and UMP) that in contrast to UDP, can penetrate into the cell, have a protective effect against myocardia ischemia, which was created by 60 min coronary artery occlusion. Both precursors significantly decreased the ischemic alteration zone in myocardium within the occlusion. The fact that glibenclamide and the specific inhibitor of mitoK_{ATP} –5-hydroxydecanoate- abolished the anti-ischemic effect of uridine and UMP indicates that the mitoK_{ATP} plays a crucial role in the antiischemic cardioprotective action of the studied preparations. Moreover, it was found that the channel is more active in high-resistant to hypoxia rats in compare with low-resistant rats and adaptation of the low-resistant rats to hypoxia by intermittent normobaric hypoxia leads to activation of mitoK_{ATP} and K^+/H^+ exchanger in mitochondria.

P20

Involvement of lipid rafts in ethanol-induced oxidative stress.

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The aim of this study was to know whether early biochemical events at the membrane, especially at lipid rafts, sphingolipid and cholesterol-rich membrane microdomains, were involved in ethanol-induced oxidative stress. To elucidate this issue, primary rat hepatocytes were incubated with lipid raft disrupters (methyl β-cyclodextrin or cholesterol oxidase). We first showed that these disrupters protected from ethanol-induced oxidative stress by the inhibition of ROS formation and lipid peroxidation. Second, membrane lipid rafts were evidenced by fluorescence microscopy. We observed that ethanol induced lipid raft aggregation and that 4-methylpyrazole (an ethanol metabolism inhibitor), methyl β-cyclodextrin and various antioxydants blocked this aggregation. Thus, oxidative alteration in lipid rafts may explain this aggregation : protein oxidative changes in lipid rafts were detected by EPR using a thiol-specific protein spin label (MAL-6) and an increase in formation of adducts with malondialdehyde, product of degradation of oxidized polyunsatured fatty acids, was quantified by HPLC-UV. In addition, using a membrane stabilizing agent (UDCA) or a membrane fluidizer (A_2C), the aggregation of lipid rafts was shown to be dependent on the increase in membrane fluidity due to ethanol. To conclude, membrane structure, depicted by membrane fluidity and lipid rafts, seems to play a key role in ethanol-induced oxidative stress in hepatocytes.

P21

The effect of vitamin E-derivative on the biochemical properties of cardiomyocytes' plasma membranes under oxidative stress

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The purpose is to study the influence of vitamin E-derivative with shortened to C6 side chain (C6) (metabolite of a-tocopherol) on biochemical and functional state of cardiomyocytes' plasma membranes (CPM) of cholesterol-fed rabbits (hypercholesterolaemia, HC).
Levels of cholesterol and free fatty acids were increased and phospholipids level was decreased in CPM under HC. Proposed mechanism of these changes is intensification of free-radical oxidation (FRO), accompanied by significant accumulation of diene conjugates, malondialdehyde, and intensification of spontaneous and induced chemiluminescence of CPM. At the same time Na+, K+-ATPase activity and speed of Na+/Ca2+-exchange of CPM under HC were decreased. HC was not accompanied by changes of catalase and superoxide dismutase activity. But the glutathionreductase activity was decreased.
The normalization of lipid structure, decrease of intensity of FRO, prevention of Na+, K+-ATPase activity and Na+/Ca2+-exchange, increase of glutathionreductase activity were found under conditions of supplementation of C6 with therapeutic and prophylactic purpose per os at the concentration of 5 mg/kg for 30 days to animals with HC. C6 provides protection against the oxidation stress. We suggest that modification of molecule's side chain modulates high effectiveness of C6.
These results prove antioxidant properties and effective membrane-stabilizing activity of C6 and provide ground for using of C6 as an adaptogene for prophylaxis and treatment of cardiovascular pathologies and obtaining the new generation of vitamin E drugs.

P22

Stabilization of IGFBP-1 mRNA by ethanol in hepatoma cells involves the JNK pathway

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Insulin-like growth factor-binding protein-1 (IGFBP-1) modulates cell growth and metabolism. IGFBP-1 induction is suggested to be a physiological mechanism to restrict growth process under stress conditions in order to preserve the energy for survival functions. The aim of our study was to determine the molecular mechanisms involved in IGFBP-1 upregulation by ethanol. The human hepatoblastoma HepG2 cell line do not expressed ethanol-metabolizing enzymes. Exposure of these cells to varying concentrations of ethanol (35 to 150 mM) induced the IGFBP-1 mRNA and protein up to 5-fold in a dose-dependent manner. A similar effect was observed using primary cultures of human hepatocytes. This effect of ethanol in HepG2 cells was not prevented by various inhibitors of ethanol metabolism and by the anti-oxidant N-acetylcysteine. While ethanol did not modify the IGFBP-1 gene promoter activity, it triggered a 2- to 3-fold increase in the IGFBP-1 mRNA half-life and this stabilisation required the 5' and the 3' untranslated region of the mRNA. Ethanol elicits a rapid and transient activation of JNK in HepG2 cells and IGFBP-1 induction was partially prevented by a specific inhibitor of the JNK pathway. This study reveals a novel pathway of gene regulation by ethanol which involves the activation of JNK and the consequent mRNA stabilisation. These data improve the current understanding of the mechanisms involved in the control of gene expression by ethanol.

P23

Free Radical homeostasis under varying alcohol intoxications and its correction by L-Arginine

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In our experiment we are make an attempt to investigate state of free radical processes under varying alcohol intoxications, to evaluate role of the adaptation to damaging ethanol action in free radical homeostasis formation and clear up influence of the L-arginine injection on above mentioned processes.

Material and methods

Experiments were performed on male Wistar rats (180g). Four groups of the animals (n-8) were used. Ethanol was administrated in drug dose (4 g/kg, intragastrically, 25% solution) twofold per day, according to schedule: I group -ethanol uninterruptedly during 56 days, II group-ethanol 7 days take turns 7 days of withdrawal, during 56 days, III group-ethanol 7 days take turns with 7 days L-arginine (intragastrically 500 mg/kg per day). Control was injected 0.95% NaCl. Animals were sacrificed after 24 hours after last drug administration. In the liver homogenates the activities of superoxide dismutase, catalase (**CAT**), glutathione system, thiobarbituric acid-reactive substances (**TBARS**) and nitrite (**NOxliv**) were measured. The activity of gamma-glutamyltranspeptidase(**GGTP**), alanineaminotransferase (**ALT**) asparagineaminotransferase (**AST**), level of lipids (**L**), concentration of nitrite (**NOxpl**) in plasma were determined.

Results

In the I group were activated free radical processes (glutathione peroxidase (GSHPO) (170%)) and activity of markers enzymes of the ethanol injury -GGTP (200%), ALT (130%) whereas NOxpl was decreased (60%). In the II group CAT and GGTP were activated up to 118% and 170% accordingly but AST was decreased. NOxliv was increasing (120%). Animals which treated by L-arginine (group III) had activated CAT (117%) and GSHPO (210%) but NOxpl, NOxliv, GGTP, AST, ALT were normalized and TBARS strong diminished (60%).

Conclusion

This results suggest that the uninterruptedly ethanol intoxication cause strong oxidative damage of the liver. Interrupted ethanol treating cause developing of the adaptation to damaging ethanol action, which may be associated with the fluctuation NO level. Treatment by L-arginine was connected with the restoration of hepatocyte membranes damaged by ethanol, regulation of the activity antioxidant enzymes and plasma and liver NO level.

P24

Effect of the perfluorocarbonic compounds (perftoran) on the antioxidant status in the liver and nitric oxide level of rats under ethanol intoxication

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Perftoran(PF) (perfluorocarbon compound) known as "blue blood" exhibited immunomodulating, antioxidant, membrane-stabilizing and disintoxicating properties. We evaluated whether PF can be used for the correction of metabolic changes in the liver caused by chronic ethanol administration.

Material and methods

Experiments were performed on male Wistar rats (180g). Animals fed ethanol in drug dose (3.5 g/kg, intragastrically, 25% solution) twofold per day during 42 days. I group was sacrificed after 1 day, II-III groups were sacrificed after 7days following the last alcohol injection. During the last 7 days of experiment, animals of II group were two fold PF injected (1ml/100 gr. i.v.), III group were two fold NaCl 0.09% solution (injected 1ml/100 gr.i.v.).
In the liver homogenates the activities of superoxide dismutase, catalase (CAT), glutathione peroxidase, alanineaminotransferase, level of glutathione, thiobarbituric acid-reactive substances (TBARS) and nitrite (NOx) were measured. The activity of gamma-glutamyltranspeptidase(GGTP), level of nitrite (NOx) in plasma was determined using Greiss reagent assay.

Results

The animals of I and III group have strongly pronounced oxidative stress (free radical processes activated, increased level of TBARS, greatly activated activity of GGTP). PF slightly inhibited CAT activity, returned to normal level of lipid peroxidation and activity of GGTP, but strong reduced NOx content both in liver and plasma.

Conclusion

This results suggest that the double intravenous PF application had promoted decrease of active forms of oxygen and the restoration of hepatocyte membranes which damaged by chronic ethanol administration.

P25

Betanin inhibits icam-1 expression in endothelial cells co-cultured with activated HL-60 cells.

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Bioactive phytochemicals, including betalain pigments such as betanin, have recently attracted interest because of their redox properties, that allow them to act as antioxidants and/or to affect redox-mediated cell pathways (1). According to recent evidences from our group (2), physical cell-cell interaction between neutrophil-like cells (HL-60 cells) PAF-activated and endothelial cells (ECs) causes intracellular generation of reactive oxygen species, and induces signalling events resulting in the over-expression of the adhesion molecule ICAM-1. The potential protective activity of betanin has been investigated in this EC dysfunction model system.
The addition of betanin to HUVECs co-cultured with activated HL-60 cells caused a marked inhibition of ICAM-1 expression. The effect was dose-dependent with an apparent IC_{50} around 5 μM, and a maximal inhibition (75%) at the highest concentration tested (20 μM). Coupled to previous findings (3), these results highlight the ability of betanin to effectively attenuate EC dysfunction, and suggest potential protective activity of this pigment in inflammatory processes. Betanin might become a lead compound for innovative anti-inflammatory therapeutics with impact on tissue injury.
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P26

The role of redox-sensitive transcription systems in the enhancement of leukemic cell differentiation

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1alpha,25-dihydroxyvitamin D_3 ($1,25D_3$) is a powerful myeloid leukemia differentiation agent, but it induces hypercalcemia at pharmacologically active doses. We demonstrated that plant-derived polyphenolic antioxidants, such as carnosic acid (CA), markedly potentiate the differentiating action of low, non-toxic concentrations of $1,25D_3$ in HL60 human leukemia cells. We hypothesize that CA modulate certain redox-sensitive transcription systems (e.g. antioxidant response element (ARE) or activating protein-1 (AP-1) systems) which potentiate vitamin D receptor (VDR) transcriptional activity, leading to the enhanced differentiation response. The goal of this study was to investigate the effects of CA, $1,25D_3$ and their combination on the ARE transcription system in myeloid leukemia cells and to determine its involvement in differentiation. We found that CA induced a strong concentration-dependent ARE transactivation and increased the levels of ARE-regulated proteins. To estimate the role of the ARE system in CA/$1,25D_3$-induced differentiation, U937 and HL60 cells were stably transfected with the major ARE activating transcription factor, Nrf2 or its dominant-negative mutant (dnNrf2). The upregulation or downregulation of the Nrf2/ARE system resulted in a significant increase or inhibition of the potentiating effect of CA. The above effects on the differentiation may be due to the observed modulation of VDR protein levels. Interestingly, dnNrf2 transfectants also demonstrated reduced levels of the AP-1 protein components, such as c-Jun and ATF2. In conclusion, our findings indicate that plant polyphenols, particularly CA, may potentiate leukemia cell differentiation induced by low concentrations of $1,25D_3$ via activation of Nrf2/ARE and probably other redox-sensitive transcription systems.

P27

Selenite is a superior cytotoxic agent to human primary leukemia cells

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The selenium compound, selenite, is rising as a promising cancer therapeutic agent in several experimental studies. However, the mechanism of selenium-induced cytotoxicity is poorly understood. This study was conducted on an *ex vivo* model with acute myeloid leukemia (AML) patient material. The primary cells were treated in a drug panel with conventional cytotoxic drugs, and evaluated in comparison to selenite treatment (5 μM). AML is the most common leukemia in adults but the cure rate remains low. Commonly used drugs, as cytarabines and antracyclines, often lead to drug resistance. We show that selenite is the most effective drug in the panel compared to commonly used drugs against AML in concentrations that could potentially be administered to patients. Equally important, all conventional drugs in the panel showed a correlation to each other by having an effect on the same group of patients. Selenite does not show this correlation indicating the ability to treat an, in part, unique group of patients. mRNA and protein levels of thioredoxin reductase and mRNA levels of the glutaredoxins were also measured While a strong upregulation of thioredoxin reductase mRNA levels were observed, the protein level decreased. This possible translational impairment may explain a part of selenites cytotoxicity. Both glutaredoxin 1 and 2 mRNA levels increased suggesting both mitochondrial and cytosolic oxidative stress caused by selenite treatment.

P28

Selenite mediated cytotoxisity in human lung cancer and the role of Thioredoxin reductase 1

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The human selenoenzyme thioredoxin reductase 1 (TrxR1) is a very important enzyme for cell growth, differentiation, and the defense against oxidative stress. Several studies have shown that TrxR1 is upregulated in tumor cells and it is a target for many anti-cancer drugs. The regulation of TrxR1 is very complex and involves the expression of different transcript forms of mRNA. We have, by quantitative polymerase chain reaction, investigated the total expression of TrxR1 mRNA and quantified the expression of alternative mRNA forms in five different human lung cancer cell lines. IC_{50} values for selenite were determined for the different cell lines and compared to the sensitivity towards doxorubicin. The results indicated an inverse relationship between resistance towards doxorubicin and selenite induced cytotoxicity. In addition, inhibition of TrxR resulted in enhanced selenite cytotoxicity. Selenium treatment resulted in increased expression of almost all TrxR1 mRNA variants while the TrxR protein activity decreased. Total TrxR1 and the less abundant forms were detected in human tissue samples from both squamous and adenocarcinoma from lung, using specific peptide antibodies. Expression of TrxR1_v.2, 3, 5 isoforms and Trx1 in the tumor correlated with degree of differentiation. Our results show that TrxR1 is involved in selenite mediated cytotoxicity and investigation of alternative transcript variants of TrxR1 could further be a valuable tool in the diagnostics and characterization of tumors.

P29

NADPH oxidases activity : regulation by fatty acids and heme oxygenase 1.

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NADPH oxydase (NOX) consists in a multimeric enzyme with a catalytic unit, gp91phox, and several regulating subunits: p22phox, p40phox, p47phox, p67phox. This enzyme, also known as flavocytochrome b588, is responsible for a deliberate production of superoxyde anion $O_2^{\cdot -}$. Initially described in polynuclear neutrophiles (NOX 2), this enzyme belongs to a complex family of multimeric isoenzymes whose members are present in many cell types. NOXs are generally associated to cell signaling and they seem involved in physiological phenomena (vascular reactivity, proliferation and cellular migration…) as well as in many diseases. Lipids in general and poly unsaturated fatty acids (PUFA) in particular are able to modulate the activity of NOX in many models.
In a model of human fibroblasts in culture, we showed that many PUFA, like arachidonic acid (AA, 20:4 n-6), docosahexaenoic acid (DHA, 22:6 n-3) or eicosapentaenoic acid (EPA, 20:5n-3), are able to induce a production of reactive oxygen species (ROS) by NOX 4. Unexpectedly, only AA was able to activate the enzyme directly in cell lysates. We also showed that the decrease of ROS production by NOX 4 in fibroblasts triggered by PUFA did not depend on SOD activity and that the time course of this decrease was associated with the expression of heme oxygenase 1 (HO-1) [1].
These observations have recently led us to propose a loop of regulation for NOX. On one side, lipids or PUFA, by interaction with phospholipase A_2 (PLA_2), could release AA which stimulates NOX, amplifying superoxyde anion production. On the other side, in addition to a regulation by protein subunits, NOX activity could be inhibited by HO-1 [2] as this enzyme, coded by a redox-sensitive gene, can inhibit NOX activity by heme degradation and CO production.
To confirm this hypothesis, we investigated the effect of arachidonyl trifluoromethyl ketone (ATK), a specific PLA_2 inhibitor. On cell lysates, ATK at 6 µM neither changed NOX activity nor modified the effect of AA at 6 µM (386 ± 79 RLU for AA alone and 387 ± 96 RLU for AA and ATK). On the opposite, ATK strongly inhibited ROS production by whole cells, as demonstrated by flow cytometry analysis of hydroethidine fluorescence of fibroblasts triggered 2 hours with 15 µM of DHA: fluorescence decreased from 171 ± 31 % to 114 ± 3 % of control in presence of 6 µM of ATK. These experiments confirm the role of PLA_2.
The role of HO-1, on the opposite side of this loop of regulation, was investigated by using siRNA technique. We were able to inhibit ROS production by silencing NOX 4 in fibroblasts triggered by DHA at 15 µM. mRNA expression of HO-1, after four hours, remained stable at a level of 1.10 ± 0.05 of control in siNOX 4 fibroblasts whereas this expression increased to 2.20 ± 0.24 of control in siScramble fibroblasts. These results confirm that HO-1 expression in our model is dependent on NOX 4 activity.

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P30

Lipoic acid prevents hypertriglyceridemia by down-regulating lipogenic gene expression in obese Zucker rats

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Fasting hypertriglyceridemia, defined as abnormally high blood triglyceride levels (>150 mg/dl), is an independent predictor of cardiovascular disease, thus, the control of hypertriglyceridemia is an important health issue of our time. Using the obese Zucker Diabetic Fatty rat as a model of hypertriglyceridemia, we found that dietary (R)-α-lipoic acid (LA) given for 5 weeks prevented the rise in blood triglycerides. This effect was not due to the appetite lowering properties of LA, as pair-fed rats developed hypertrigly-ceridemia. Dietary LA inhibited hepatic de novo synthesis of fatty acids and triglycerides by down-regulating expression of acetyl-CoA carboxylase, fatty acid synthase, glycerol-3-phosphate acyl-transferase, and diacylglycerol acyltransferase. Liver glycogen content was increased by LA, suggesting that LA induced dietary carbohydrates to be stored as glycogen. Liver AMP-activated protein kinase, a previously recognized mediator of LA, did not take part in these effects. Moreover, we found no evidence that PPARα-dependent hepatic β-oxidation was stimulated by dietary LA. LA-fed rats were leaner, exhibiting less perivisceral fat deposits than pair-fed animals. Glycemia and insulinemia were not significantly different among the treatments. Our results indicate that LA is not just an appetite-lowering compound, but also has a true metabolic effect on triglyceridemia independently of caloric intake, AMP-activated protein kinase, and PPARα. We have uncovered a novel means of controlling hypertriglyceridemia with potential application in human health.

P31

Effect of dietary supplement DiavitÒ on antioxidant capacity in diabetes mellitus disease

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Background. It is now well recognised that diabetes mellitus is a major worldwide health problem. Long-term complications are main cause of morbidity and mortality. Recent studies demonstrate that hyperglycemia-induced overproduction of superoxide seems the first and key event in the activation of all pathways involved in the pathogenesis of diabetic complications. Elaboration of efficient strategies for the prevention and the control of this disease represent at present an priority for the international scientific community.
Aims. The aim of this study was to investigate the relationship between the level of glycated hemoglobin and changes in the status of two antioxidant enzymes, in type 1 diabetic children treated with Diavit® (a *Vaccinium myrtillus* and *Hippopphae rhamnoides* extract). We also investigated the effect of Diavit® treatment in streptozotocin diabetic-rats.
Materials and methos. The study included 14 children suffering from type 1 of diabete (age 12,59, SD=3,65). Erythrocyte superoxide dismutase (SOD) activity and whole blood glutathione peroxidase (GPx) activity were determined using RANSOD and RANSEL kit (Randox Laboratories Ltd. UK). Assay of glycated hemoglobin was performed by an immunoturbidimetric method on the Konelab Analyser.
Results. The erythrocyte SOD activity was significantly high ($p<0,05$) in diabetic children after two month of Diavit® administration and the levels of glycated hemoglobin were significantly smaller ($p<0,05$). The activity of whole blood GPx was moderately increased but the difference is not statististically significant. Studies carried out in streptozotocin diabetic rats demonstrated that the treatment with Diavit®, for two month, has a regenerative effect on pancreatic beta cells.
Conclusions. These results suggests that treatment with Diavit® has a benefic effect in type 1 diabetic children and this phytotherapeutical product seems to be an interesting therapeutic prospect for the prevention of diabetic complications.

P32

Tocotrienol and a-Tocopherol: comparative distribution and effect on oxidative status in stress-induced rat's organs

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This study was designed to investigate the effects of tocotrienol (TT) and tocopherol(TF) supplementation on oxidative status and the distribution OF this antioxidant vitamin in stress-induced rats. 60 male *Sprague-Dawley* rats were randomly assigned into four equal sized groups, two control groups (C and CS) were fed with a normal rat diet while two treatment groups received the same diet but with supplementation of tocotrienol mixture (TT) or α-tocopherol (TF) orally at the dose of 60mg/kg body weight. After 28 days of treatment, the CS, TT and TF rats were subjected to restraint stress, two hours daily for four consecutive days. The rats were killed and the main organs such as liver, heart and kidney was taken to determine malondialdehyde (MDA) level and HPLC was done to measure the distribution of TT and TF in these organs. The findings showed that CS rats that had been exposed to stress had significantly higher levels of MDA in liver, heart and kidney compared to the pre-stress values. TT and TF were also proved to significantly reduced the content of MDA in tissues after exposure to stress compared to control groups. All isomers of TT were detected in the group receiving tocotrienol and the distribution is the highest in the liver but with similar distribution observed in the heart and kidney. While TF was distributed mainly in the kidney followed by the liver and heart. Stress causes an increased in the distribution of both TT and TF in these organs. In conclusion, tocotrienol and tocopherol are capable in reducing the oxidative stress by reducing MDA tissue level and this correlates with the findings that an increased distribution of TT and TF observed in stress-induced rats.

P33

Bowman-Birk Inhibitor Concentrate Protects Soleus Muscle From Atrophy, Weakness And Oxidative Stress Induced By Prolonged Unloading.

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Prolonged mechanical unloading induced by bedrest or space flight are known to cause antigravity muscles atrophy and weakness. Unloading also induces oxidative stress in muscle, a putative cause of weakness. We hypothesized that dietary supplementation with Bowman-Birk Inhibitor Concentrate (BBIC), a soy protein extract, would protect soleus muscle against those changes. Adult mice were fed a diet supplemented with 1% BBIC during hindlimb unloading for up to 12 days. After unloading, soleus muscles weighed less, developed less force per cross-sectional area, and developed less total force relative to controls. BBIC supplementation was protective, blunting decrements in soleus weight and force. Cytosolic oxidant activity was assessed using 2',7'-dichlorofluorescin diacetate. Oxidant activity increased in unloaded muscle, peaking at day 3 and remaining elevated through day 12. Increases in oxidant activity correlated directly with loss of muscle mass and were abolished by BBIC supplementation. *In vitro* assays established that BBIC directly buffers reactive oxygen species and also inhibits serine protease activity. We conclude that dietary supplementation with BBIC protects skeletal muscle during prolonged unloading, promoting redox homeostasis in muscle fibers and blunting atrophy-induced weakness.

Key Words: skeletal muscle, cachexia, free radicals, oxidative stress, antioxidant, microgravity, nutrition

P34

a-Tocopherol antioxidant resource in muscle tissue of *Engraulis encrasicolus*

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In the present work the effect of chilling storage, salting, marinating and frying was analysed by HPLC on a-tocopherol levels in *Engraulis encrasicolus* muscle tissue. From chilled fish muscle, the left fillet was utilized as fresh fillet, while the right fillet was stored for 24h and 48h at 4°C before analyses. Salting and marinating was performed only on fresh fillet, while frying on fresh fillet and fillet after storage for 24h and 48h at 4°C. Our results show the a-tocopherol resource decreases significantly during storage. A comparison of a-tocopherol levels between salted, marinated and fresh fish demonstrates no significant differences, while a comparison of a-tocopherol levels between fried and fresh fish indicates that the a-tocopherol level in fried, decreases significantly. The relative amount of a-tocopherol could be associated with potential lipid peroxidation in post-mortem fish, and considered as an indirect index of oxidative stress effects. Salting and marinating in *Engraulis encrasicolus* suggest the possibility of good food preservation methods for maintaining the high antioxidant resource of a-tocopherol.

P35

Protective effect of aqueous crude extract fraction of *Euphorbia hirta* L. leaves on oxidative damage to biomolecule.

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The aqueous crude extract of *Euphorbia hirta* L fresh leaves was prepared in boiling water. Crude extract was further fractionated with liquid-liquid partitioning method into n-hexane, diethyl ether, ethyl acetate, methanol, ethanol and water fractions. The results obtained from DPPH (2, 2'-diphenyl-1-picrylhydrazyl) free radical scavenging activity assay revealed that only ethyl acetate and methanol fraction showed most of the scavenging activity with EC_{50} concentration 20 and 14 µg per ml extract fraction, respectively. The deoxyribose degradation experiment was carried out using hydroxyl radical generated from iron mediated Fenton reaction. The results revealed significant hydroxyl radical scavenging potential with EC_{50} 108 and 86 µg per ml extract fraction for ethyl acetate and methanol fraction, respectively. The ethyl acetate and methanol fractions didn't show pro-oxidant activity in the absence of reducing agent. The protective potential against oxidative damage to calf thymus DNA and plasmid was studied on 1% agarose gel using copper (II) and ascorbate reaction mixture as source of free radical damage. The densitometric analysis of DNA band intensity was performed to study the extent of protection against control DNA damage. The results showed marked inhibition potential against oxidative damage to DNA with EC_{50} 120 and 93 µg per ml extract fraction for ethyl acetate and methanol fraction, respectively. The protective effect of ethyl acetate and methanol fraction on hypochlorite mediated oxidative damage to bovine serum protein was studied based on native PAGE and spectrophotometer technique. The results revealed that both fractions ethyl acetate and methanol showed significant inhibition potential with EC_{50} 220 and 158 µg per ml extract fraction, respectively. On the basis of above findings, we found these two fractions ethyl acetate and methanol as good source of antioxidant activity and convincing protective role against oxidative damage to DNA and protein.

Key Words. Free radical, antioxidant, scavenging, oxidative damage, hydroxyl radical

P36

Antioxidant activity of *Eupatorium Inulifolium* H.B.K (an Indonesian medicinal plant; local name "*Ki-Rinyuh*") and its active compound

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Antioxidant activity study of *Eupatorium inulifolium* H.B.K. (Asteraceae) leaves, an Indonesian medicinal plant, with local name "Ki-rinyuh", had been carried out. Extract had been prepared using ethanol by maceration and percolation method, and the extract was partitioned into n-hexane, EtOAc and aqueous fractions. Antioxidant activity study had been done using DPPH-TLC Antioxidant Assay and The Superoxide Radical Scavenging Activity Assay.
Both EtOAc and aqueous fractions showed significant antioxidant and from the EtOAc fraction an active compound, called EI-2 had been isolated. The EI-2 was identified as a flavonoid Guajaverin (3-O-L-arabinosylquercetin) based on its UV-Vis, IR, H-NMR spectra.

P37

Antileukemic activity of plant polyphenolic antioxidants

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Acute myeloid leukemia (AML) is the most common acute leukemia in adults, for which there is no effective therapy at this time. Differentiation therapy with vitamin D_3 derivatives (VDDs) is a promising potential approach to treat AML. However, most VDDs induce severe hypercalcemia at pharmacologically active doses. We found that plant polyphenols, such as carnosic acid, curcumin and silibinin inhibit proliferation of AML cells and synergistically enhance the differentiation effects of very low VDD doses, without inducing cytotoxicity. These effects were associated with a decrease in the intracellular levels of reactive oxygen species, modulation of redox- and differentiation-related transcription factors, and elevation of glutathione content. In the *in vivo* study, we tested the hypothesis that the synergy between polyphenols and VDDs can be exploited for differentiation therapy of AML. The leukemic tumor and systemic AML models in syngeneic Balb/c mice were established by intraperitoneal (i.p.) and intravenous, respectively, inoculation of WEHI-3B D^- murine myelomonocytic leukemia cells. The tumor- or systemic leukemia-bearing mice were separately treated with polyphenols (carnosic acid-rich rosemary extract or silibinin; mixed with food), low-calcemic VDDs (nor-gemini, 1,25-dihydroxy-21(3-hydroxy-3-methyl-butyl)-19-nor-cholecalciferol or 1,25-dihydroxy-20,16-ene-5,6-trans-cholecalciferol; i.p.) and their combinations. The combined treatments resulted in at least an additive strong inhibition of tumor growth as well as in a substantial increase in the life span of the systemic leukemia-bearing mice. These cooperative effects of polyphenols and VDDs in vivo were not accompanied by hypercalcemia or general drug toxicity. Our results suggest novel therapeutic and preventive strategies against AML and may have a carry-over significance in other types of cancers.

P38

Curcumin: a therapeutical agent in Hodgkin's lymphoma?

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Both NF-?B and STAT-3 transcription factors are constitutively active in Hodgkin's lymphoma. As the deregulation of NF-?B and STAT-3 occurs in different types of cancer, this has intensified the interest in finding molecules that could inhibit NF-?B or STAT-3, as a therapeutically strategy against cancer. Curcumin have been shown to inhibit NF-?B- and STAT-3 activation and the expression of both NF-?B- and STAT-3-regulated genes in different cell lines. Since Hodgkin and Reed-Sternberg (H-RS) cells present constitutive NF-kB and STAT-3 activation, we investigated the capacity of curcumin to inhibit NF-?B and STAT-3 in H-RS cells, characterizing the functional consequences. Curcumin inhibited both NF-κB and STAT-3 activation leading to a decreased expression of proteins involved in cell proliferation and apoptosis (Bcl-2, Bcl-xL, FLIP, XIAP, c-IAP1, survivin, c-myc, cyclin D1). After 72 h, curcumin caused a significant reduction (80-97%) in H-RS cell viability. This was associated with the triggering of cell death by apoptosis, as evidenced by the activation of caspase-3 and caspase-9, nuclear morphology, and Annexin-V staining,
Thus, curcumin merits further evaluation as a chemopreventive agent against Hodgkin's lymphoma.

P39

Antimitotic and Antioxidant Effects of Alcaloids from *Solanaceae* and Triterpenes from *Ericaceae.*

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In the present paper we report the antimitotic and antioxidant effects of two natural products: hydroalcoholic extract from *Solanum nigrum* L. and *Calluna vulgaris* L. *Solanum nigrum* is a specie in the *Solanaceae* family, that contains glycoalcaloids, the most important being solasonine, solamargine and tropane alkaloids . *Calluna vulgaris* belong the *Ericaceae* family, and contain triterpenes, where the ursolic acid ursolic acid is the principal component.

These products antioxidative effects were measured using the stable radical DPPH spectrophotometric assay, in serial dilutions at 5 and 60 minutes. While *Solanum nigrum* had no detectable antioxidant capacity, *Calluna* extract, which contains polyphenols, exhibited a dose dependent antiradical capacity.

Toxicity of these extracts was evaluated using the viability test described by Mossman (MTT test). The inhibitory effect of *Solanum nigrum* and *Calluna vulgaris* tested on two cultures cell lines, normal fibroblast Hfl-1 and highly malignant M1/15 tumor cells, at 24 and 48 hours, demonstrated higher cytotoxic activity in tumor cells that in normal ones.

The values obtained from these dose-effect sigmoid curves helped us to select appropriate concentrations of extract in the associated treatments with Doxorubicin, a potent antitumoral drug known to cause severe cardiotoxicity due to free radicals production. Therefore, we evaluated the influence of Calluna vulgaris extract supplementation on the cytotoxicity induced by this antineoplastic drug. In this concern, our results showed that the Calluna vulgaris extract exhibited cytoprotective effect in normal Hfl-1 cells, whereas both natural preparates synergically enhanced the inhibitory effect in a dose-effect relationship. These data demonstrate potential use of extract in pretreatments with antineoplastic drugs.

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Protective Effects of a Grape-seeds Hydroalcoholic Extract (BMR) in the Treatment with Doxorubicin

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Doxorubicin is a broadly used agent to treat a variety of malignancies, but the main limitation of its clinical use is the development of cardiac toxicity. This phenomenon is subsequent to generation of reactive oxygen species and leads to some severe cellular damages (lipid peroxidation, DNA fragmentation).
Antioxidant product isolated from fruits and plants might be potential candidates to fight these side-effects in an effective antioxidant therapy.
We report here some "in vitro" protective properties of a grape-seeds hydroalcoholic extract of *Vitis vinifera* (variety Burgund Mare, Recas, Romania). Thus, when given in association with Doxorubicin (30 min. before cytostatic administration), in human fibroblasts (Hfl1) cell line, BMR showed a protective effect assesed from IC50 values measured by MTT test. On contrary, against cervix carcinoma cell line (HeLa), the Doxorubicin inhibitory activity was enhanced by BMR pretreatment. In both cases, the magnitude of the effects was dependent on the extract' concentrations. Further tests (lipid peroxidation, metalloproteinases 2 and 9 release) on this product are now in progress.

P41

Antioxidant, anticomplementary and TNF-a Inhibitory activity of poly[3-(3,4-Dihydroxy-Phenyl) glyceric acid] from *Symphytum Officinale*

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Hot water extraction of roots of *Symphytum officinale* afforded crude polysaccharides C-SOP (yield 12.4% of dry biomass). Further ultrafiltration of C-SOP on membrane filters with cut-off values of 1000 kDa gave water-soluble high-molecular (>1000 kDa) fractions SO-HMF. According to IR, ^{1}H and ^{13}C NMR and 2D heteronuclear $^{1}H/^{13}C$ HSQC spectral data the main chemical constituent of SO- HMF is a regular caffeic acid-derived polymer poly[3-(3,4-dihydroxyphenyl)glyceric acid] or poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene]. SO- HMF was tested in several *in vitro* assays: 1) inhibition of human complement system; 2) inhibition of reactive oxygen species produced by opsonized zymosan-stimulated human polymorphonuclear neutrophils (chemiluminescence assay); 3) scavenging of superoxide anions generated in a cell-free hypoxanthine/xanthineoxidase system (chemiluminescence assay); 4) inhibition of inflammatory cytokine tumor necrosis factor-α (TNF-α) production by adherent human mononuclear cells and exhibited anticomplementary, antioxidant and TNF-α inhibitory activities. Our results show that the *S. officinale* poly[3-(3,4-dihydroxyphenyl)glyceric acid] might be a protective/therapeutic agent in free radical-induces and/or enzyme-related inflammatory and vascular diseases and thus has valuable therapeutical potential.

P42

Stability and antioxidant capacity of food polyphenols in two chemical models of the gastric tract.

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Diets rich in fruits and vegetables are associated with a lower incidence of cardiovascular and degenerative diseases. Dietary antioxidants, such as polyphenols, are possible mediators of these beneficial effects. Indeed polyphenols could play a key role within the gastro-intestinal tract, where they are accumulated in large concentrations after a meal rich in plant products and where lipid peroxidation induced by dietary iron is quite fast. The main goal of this work is the *in vitro* investigation of lipid oxidation processes possibly taking place in the gastric tract and their inhibition by food polyphenols. Modelling of the gastric conditions is achieved by sonicating sunflower oil in mildly acidic buffer in presence of albumin or phospholipids. Lipid oxidation of the model emulsions is initiated by metmyoglobin, a common form of dietary iron (from red meat), and assessed by the spectroscopic measurement of dienylhydroperoxides. Some dietary abundant polyphenols (quercetin, rutin, (+)-catechin, caffeic acid, chlorogenic acid) are evaluated for their capacity to inhibit heme-initiated lipid oxidation in the model gastric emulsions. Consumption of polyphenols and formation of the degradation products are followed by HPLC-UV and HPLC-MS. Results indicated that all the tested polyphenols inhibited lipid peroxidation in concentrations found in food (25-100 µM). Quercetin proved to be the best inhibitor in both models. Phospholipid-stabilised emulsions were less strongly inhibited by food polyphenols compared to albumin-stabilised ones outlining the influence of lipid droplet sizes and polyphenols interactions with albumin. Structure elucidation of the polyphenol degradation products revealed the formation of several oxidation products that are themselves potent inhibitors.

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Metalloporphyrins, from antioxidants to photosensitizers

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Mn(III) porphyrins (MnTM-2-PyP^{5+} and MnTE-2-PyP^{5+}), initially designed as SOD mimics, can catalytically scavenge hydrogen peroxide, peroxynitrite, carbonate radical, nitric oxide, and lipid peroxyl radicals. They protected SOD-deficient cells against oxidative damage and proved beneficial in sickle cell disease, cancer, stroke, and spinal cord injury. In contrast, Zn(II) *N*-alkylpyridyl-porphyrins (ZnTMPyP and ZnTEPyP), lacking accessible redox chemistry at the metal site, are inactive as antioxidants. Upon illumination, however, they kill antibiotic-resistant bacteria and tumor cells. The mechanism of cell killing depends upon the availability of oxygen. In the presence of oxygen the cells showed morphological changes typical for necrosis, while hypoxic cells demonstrated nuclear fragmentation typical for apoptosis. Light exposure of aerobic, ZnTMPyP-treated cells, causes oxidative stress, manifested by depletion of GSH, production of 8-oxo-deoxy guanosine and peroxidation of membrane lipids. In addition, ZnTMPyP damages membranes by crosslinking membrane proteins, and suppresses cell metabolism and antioxidant defense by photo-inactivating metabolic and antioxidant enzymes. A unique feature of ZnTMPyP, compared to other photosensitizers such as hematoporphyrin D, is that it inactivates enzymes and induces protein crosslinking not only aerobically but also anaerobically. Keeping in mind that most aggressive tumors are hypoxic, which limits the efficacy of most known photosensitizers, this property of ZnTMPyP is potentially of great advantage.

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Modulation of arterial blood pressure by a calibrated oxidant load in preterm infants.

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Background: Shielding total parenteral nutrition (TPN) from light protects against the generation of organic & inorganic peroxides. In situ, we found that these peroxides cause vasoconstriction. We hypothesized that modifying the oxidant load in TPN is associated with clinical consequences.
Objective: Determine in neonates if shielding TPN from light modulates blood pressure.
Methods: 40 preterm infants < 1000g with arterial catheter were randomized to light exposed (LE) or light protected (LP) TPN. Arterial blood pressure (BP) was recorded hourly and compared between LE and LP over week 1 of life. A subgroup (LE=7, LP=12) had timed average maximum velocity (TAmax, m/s) measured in the superior mesenteric artery (SMA) by Doppler. The ductus arteriousus was detected by cardiac ultrasound. Data (mean±SEM) were analyzed by ANOVA.
Results: No differernces in demographics, severity of illness, vasopressors, fluid or nutrient intakes between LE & LP. There was an interaction ($p<0.01$) between gender & TPN on BP and a correlation between TAmax and BP ($p<0.05$). In girls (n=17) receiving LE, systolic & diastolic BP were higher ($p<0.01$) & heart rate lower ($p<0.01$) compared to LP. In boys (n=23), there were no differences in blood pressure, but a higher heart rate compared to girls ($p<0.01$). The incidence of patent ductuous arterious was higher ($p<0.01$) in boys.
Conclusion: The data suggest that LE increases vascular resistence in girls, which might be explained by the oxidant load infused during LE. The lack of response in boys might be explained by the greter incidence of PDA or their known weaker antioxidant defenses. The higher BP reported after puberty in female ex premature infants might be realted to our findings in early postnatal life.

P45

Antioxidant micronutrients for oxidative and inflammatory stress in cystic fibrosis

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RATIONALE: The leading cause of morbidity and mortality in Cystic Fibrosis (CF) is chronic progressive lung disease. Airway infection leads to progressive damage of the lung tissue, due in part to oxidative stress. Further, the body's antioxidants are depleted in conditions of acute oxidative stress (Back 2004, Ciabattoni 2000, Winklhofer-Roob 1994). In CF, the source of oxidative stress is twofold – the infectious agent and the body's inflammatory immune response. Supplementation of exogenous antioxidant micronutrients (vitamin E, vitamin C, beta-carotene and selenium) may help in maintaining an oxidant-antioxidant balance. Current literature suggests a relationship between oxidative status and lung function exists, often using oxidative and inflammatory markers as surrogate outcomes (Repine 1997, Schünemann 1997, Wood 2003). A Cochrane Systematic Review (SR) will synthesize existing knowledge of the effect of antioxidant micronutrients on oxidative and inflammatory stress in CF patients.

METHODOLOGY: MEDLINE (1966-present), PubMed (1966-present), EMBASE (1988-present), the Cochrane Controlled Trials Register (1991-present) and CINAHL (1937-present) have been searched using the following search strategy: Cystic fibrosis AND vitamin E AND vitamin C AND beta-carotene AND selenium AND antioxidants (and respective synonyms). Appropriate filters were employed to guarantee the extraction of only controlled clinical trials. Primary outcomes are lung function and quality of life. Secondary outcomes include measures of oxidative stress and inflammation. Screening, data-extraction and quality assessment (using Jadad and allocation concealment) will be conducted independently by two reviewers. Meta-analysis and sensitivity analysis may be performed and if so, subgrouped by sole antioxidant supplementation versus antioxidants with concurrent treatment. Sensitivity analysis will include the following: 1) intention to treat analysis, 2) quality analysis, 3) publication bias analysis and 4) analysis based on timing of intervention (preventative vs. therapeutic) if this distinction exists.

OUTCOMES: Five hundred forty-five articles were identified from the initial search and have been screened for potential inclusion. The full-texts of 63 articles are currently being screened for final inclusion.

SIGNIFICANCE: While routine supplementation of fat-soluble vitamins addresses deficiencies in CF, the effect of antioxidant micronutrients, some of which are fat-soluble vitamins (E and ß-carotene) is still unclear. As such, the synthesis of literature on the effect of antioxidant micronutrients for lung disease in CF will provide an indication of relevance of which antioxidants, if any, are promising adjunct therapies.

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Anthocyanins inhibit NF-?B activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults

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The transcription factor NF-κB is activated by oxidative stress and pro-inflammatory stimuli, and controls the expression of numerous genes involved in the inflammatory response. Reducing NF-κB activation and thereby limiting the inflammatory response have been suggested as a potential strategy in the treatment and prevention of chronic inflammatory diseases.

In cultured monocytes we observed that anthocyanins isolated from bilberries and black currants (Medox®) efficiently reduce LPS induced activation of NF-κB. Furthermore, we studied the effect of Medox® supplementation (300 mg/day) in a parallel designed placebo-controlled clinical trial (n=120, men and women, age 40-74) for three weeks. Significant reductions were observed in plasma concentrations of several pro-inflammatory cytokines that induce, or are induced by NF-?B activation, such as IL-8 (45 % reduction in median value, P=0.020), RANTES (15 % reduction in mean value, P=0.048) and IFN-α (40 % reduction in median value, P=0.016). Additionally, trends were observed for the pro-inflammatory mediators IL-4 and IL-13 (60 % reduction in median value, P=0.056 and 38 % reduction in median value, P=0.089, respectively).

These data suggest that anthocyanin supplementation may have a role in the treatment and prevention of chronic inflammatory diseases by inhibition of NF-?B transactivation and reduction of plasma concentration of pro-inflammatory chemokines, cytokines and inflammatory mediators.

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Oxidative stress and serum activity of matrix metalloproteinases in patients with Takayasu's Arteritis Disease

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Background: Takayasu's arteritis (TA) is a chronic large vessel vasculitis and a sizeable problem in South Asian countries including India and Japan. The poor prognosis of TA despite the use of multiple evidence-based therapies has provided an impetus for the elucidation of new pathophysiological pathways that could be therapeutically targeted to improve patient's outcome. Two such pathways that have received increasing attention in other inflammatory diseases are (i) oxidative stress and (ii) activation of matrix metalloproteinases (MMPs), a family of zinc endopeptidases capable of degrading extracellular matrix components. As TA is an inflammatory disorder characterized by destruction of elastic fibers, we hypothesized that monitoring oxidative stress and MMPs could be useful as biomarkers of disease activity in TA patients. Moreover, the degree of oxidative stress and how it is related to the TA is poorly explored. Based on this background, we investigated for the first time, the correlation between oxidative stress and activity of MMPs in this disease.
Methods: 35 Patients with clinically defined TA (Group I) and equal number of healthy, age and sex-matched subjects as controls (Group II) were enrolled in the study. Patients with TA were divided into those with clear cut clinically active or in remission based on vasculitis activity score. Levels of a sensitive and specific *in vivo* biomarker of oxidative stress i.e. 8-iso-Prostagandin F_{2a} (8-iso-PGF_{2a}) was determined in plasma of all the study subjects using enzyme immunoassay. Activity of matrix metalloproteinases -2 and 9 were measured in serum by gelatin zymography.
Results: Significantly augmented levels of 8-iso-PGF_{2a} were observed in plasma of TA patients as compared to the control subjects ($p<0.05$). Also, levels of 8-iso-$PGF_{2a,}$ were found to be further significantly higher in Group I subjects with active disease as compared to those in remission phase ($p<0.05$). Zymography of serum showed bands in range of 85-92 KDa (MMP-9) and 65-72 KDa (MMP-2). The MMP activity was expressed as a ratio to MMP-9 standard. Total gelatinolytic activity was found to be significantly higher in subjects with TA as compared to the normal healthy controls ($p<0.05$).A low level of gelatinolytic activity was observed in serum of normal, healthy controls and TA subjects in remission as compared to the TA subjects in active phase of disease. Also, gelatinolytic activity showed a positive correlation with plasma levels of 8-iso-PGF_{2a} in patients with TA..
Conclusions: Increased oxidative stress and augmented MMP activity may be involved in the development of vascular remodeling in patients with Takayasu's arteritis disease and, thus make these subjects more vulnerable to the damaging effects of oxidative stress.

P48

Cholesterol peroxides as an inducer of matrix metalloproteinase activity in UVA-exposed hairless mouse skin

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Role of ultraviolet A (UVA) on the skin damage has attracted much attention in relation to photoaging process. We examined the mechanism of photoaging in UVA-irradiated skin, and found that cholesterol hydroperoxides (ChOOHs) is one of the inducers of collagen hydrolyzing enzymes, matrix metalloproteinases (MMPs) activation. By exposure of UVA for 8 weeks (total 140 J/cm^2) to male hairless mice (Hos; HR-1, 5 weeks-old), accumulation of ChOOHs was observed in the GC-MS. In addition, MMP-9 activity was elevated with the formation of the wrinkle in the skin. MMP-9 activity was also enhanced by intracutaneous injection of ChOOHs into the back skin of mice. These results suggest that ChOOHs formed by UVA irradiation, at least partly, contribute to the activation of MMPs resulting in skin photoaging. The effect of dietary carotenoids on the activation of MMP will be also presented.

P49

Involvement of oxidative DNA damage and antioxidant status in human aging

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Ageing is a complex process involving morphologic and biochemical changes in single cells and in the whole organism. One of the most popular explanations of how ageing occurs at the molecular level is the oxidative stress hypothesis. The aim of this work was to assess age-related changes in oxidative DNA damage in 255 humans, 3 – 83 years old. For the first time, the broad spectrum of oxidative DNA damage biomarkers was analysed: urinary excretion of 8-oxodG and 8-oxoGua as well as the level of oxidative DNA damage in leukocytes. In addition a concentration of antioxidant vitamins A, C, E and uric acid was determined in blood serum. The level of 8-oxodG in leukocytes' DNA showed statistically significant correlation with the age of the examined subjects, and the level of urinary 8-oxoGua and 8-oxodG followed the same pattern. Age-dependent decline in the concentration of vitamin C, a small but statistically significant increase in the level of vitamin E and gradual increase of uric acid with age was observed. On the basis of the presented correlative association between oxidative DNA damage parameters and age it seems reasonable to state that the damage may be one of the substantial factors in human ageing. Furthermore, an inverse correlation of vitamin C concentrations with age is also consistent with the oxidative stress hypothesis of ageing.

P50

Ginkgo biloba Extracts (EGb 761) Can Reduce Neurological Deficit Scores via Stimulating Neural Proliferation and Differentiation in Transient Focal Cerebral Ischaemic Rat Brains

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EGb 761, an extraction from *Ginkgo biloba* leaves, has been extensively used to protect cerebral brain tissue against ischemic vascular diseases and neurodegenerative disorders including Parkinson's and Alzheimer's diseases. EGb 761 possesses antioxidant activity and has potential to improve nerve regeneration. To explore its potential application for neural regeneration, we investigated the effects of EGB 761 on the neurological behavior performance, infarction volume and the proliferation and differentiation of progenitors in focal cerebral ischaemia rat brains. Transient focal cerebral ischemia was induced by occlusion of middle cerebral artery of Wistar rats. The neurological deficit scores and infarction volume were measured at day 3, 7, 14 and 28 after 30 min of occlusion of middle cerebral artery. EGb 761 was administrated into the rats for 14 days. The results showed that administration of EGb 761 improved significantly the neurological behavior performances and reduced the infarction volume in the ischaemic brains. We detected the immunofluorescent staining of thymidine analog 5-bromo-2'-deoxyuridine (BrdU) and other mature neural marks including NeuN, MAP-2 and GFAP in the hippocampus and subventricular zone (SVZ) of the ischaemic brains. We found that transient focal cerebral ischemia induced temperately the increase of BrdU positive cells but not induced the BrdU/NeuN and BrdU/MAP-2 double staining cells. Administration of EGb 761 significantly increased the number of BrdU positive cells as well as BrdU/NeuN and BrdU/MAP-2 double staining cells but not BrdU/GFAP staining cells. The results indicate that the therapeutic effects of EGb 761 for recovery of neurological deficits are associated with the stimulation of the proliferation and differentiation of progenitors in ischaemic brains.

P51

Buyang Huanwu Decoction Can Improve Recovery of Neurological Function via Stimulating Neural Proliferation and Differentiation and Modulating VEGF and Flk1 Expressions in Transient Focal Cerebral Ischaemic Rat Brains

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Buyang Huanwu Decoction is a classic formula for treating stroke-induced disability in Traditional Chinese Medicine (TCM). To explore its pharmacological basis, we investigated the effects of the whole formula and its herbal components on the neurological behavior performance and infarction volume in focal cerebral ischaemia rats. The neurological deficit scores and infarction volume were measured at day 3, 7 and 14 after 30 min of occlusion of middle cerebral artery. The results showed that Buyang Huanwu Decoction and its herbal components significantly improved the neurological behavior performances and reduced the infarction volume in the ischaemic brains. To elucidate the potential therapeutic mechanisms, we investigated the proliferation and differentiation of progenitors by detecting the immunofluorescent staining of thymidine analog 5-bromo-2′-deoxyuridine (BrdU) and other neural marks including NeuN, MAP-2, GFAP and found that the formula stimulated the proliferation and differentiation of the progenitors at hippocampus and subventricular zone (SVZ) in the ischaemic brains. As vascular endothelial growth factor (VEGF) and its receptor fetal liver kinase (Flk1) are important neurotrophic, neuroprotective and neuroproliferative factors, we studied the expressions of VEGF and Flk1 in the hippocampus, SVZ and cortex in the ischaemic brains and found that the formula led to increase the numbers of VEGF-positive and Flk1-positive cells in the SVZ and cortex in the ischaemic brains. The results indicate that the therapeutic effects of Buyang Huanwu Decoction for recovery of neurological deficits are associated with the stimulation of the proliferation and differentiation of progenitors and the enhancement of the expressions of VEGF and Flk in ischaemic brains.

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Antioxidant Activities of Methionine Account for the Evolution of a Non-Standard Genetic Code in Mitochondria: Implications for Future Interventions Targeting Mitochondrial Aging

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Methionine is an essential amino acid which is readily oxidized by reactive oxygen species (ROS). The main products of methionine oxidation are R- and S-methionine sulfoxide, which can be reduced by two classes of stereospecific methionine sulfoxide reductases (MSR-A and MSR-B). In order to investigate whether the methionine/MSR system constitutes an evolutionarily relevant antioxidant cycle to protect eukaryotic cells from oxidative injury, we have analyzed the usage of methionine in approximately 400 mitochondrially and nuclear encoded proteomes. We have found that methionine is strongly enriched in proteomes confronted with increased levels of oxidative stress: Mitochondrially encoded proteins of most animals and several fungi exhibit up to 5fold higher methionine contents than corresponding nuclear encoded proteins. The increased mitochondrial methionine contents are achieved by the use of a non-standard genetic code, assigning a second codon to methionine (AUA Ile -> Met). This deviant genetic coding is exclusively found in species which also seem to possess nuclear sequences coding for both MSR-A and MSR-B. Our findings suggest that the antioxidant activity of the methionine/MSR system has provided the strong selective advantage that is necessary to effectuate alterations in the genetic code. These results might provide a specific explanation for the accelerated aging phenotype observed in MSR-A knock-out mice. In addition, they point towards respiratory chain protein repair as a potential target for future mitochondrial anti-aging interventions.

P53

Effect of zinc supplementation in the elderly on oxidized protein degradation and repair systems in peripheral blood lymphocytes

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Aging has been associated with zinc deficiency, leading to chronic inflammation and subsequent oxidative stress, especially in the immune system. Proteins are very sensitive to almost all forms of reactive oxygen species and the accumulation of oxidized proteins raises the problem of the efficacy of intracellular protein maintenance systems responsible for the elimination of oxidatively modified proteins. Protein degradation and repair are critical for eliminating oxidized proteins from the cell. The degradation of oxidized protein is mainly achieved by the proteasomal system and it is now well established that proteasomal function is generally impaired with age, as previously reported for myocytes, keratinocytes and peripheral blood lymphocytes. Repair is limited to a few modifications, such as methionine oxidation, that can be reversed within proteins by the peptide methionine sulfoxide reductase A and B enzymes. MsrB are zinc-containing enzymes and metal binding has been shown to be essential for their activity. Importantly, the methionine sulfoxide reductase system has been implicated in increased longevity and resistance to oxidative stress in different cell types and model organisms. To study the role of zinc on protein maintenance systems, we analyzed the status of both proteasome and methionine sulfoxide reductases in twenty three healthy old subjects (age-range between 59-84 years old), before and after zinc supplementation (10mg of zinc per day for one month). Oxidized protein content and proteasome peptidase and methionine sulfoxide reductase activities and expression were monitored in peripheral blood lymphocytes samples of control and zinc-supplemented subjects. MsrB overexpressing MolT-4 leukemia cell line was also produced and investigated for relationships between Zinc, methionine sulfoxide reductases and oxidative stress. Our results indicate that zinc treatment could enhance the anti-oxidative defences of lymphocyte cells by increasing the activities of maintenance systems responsible for the elimination and repair of oxidatively modified proteins.

P54

Stimulation par un extrait d'algue de la dégradation de la tyrosinase par le protéasome dans des mélanocytes humains.

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La tyrosinase est l' enzyme clé de la voie de synthèse de la mélanine. La dérégulation de l'activité de la tyrosinase entraîne localement l'accumulation de mélanine dans la peau conduisant à des problèmes de pigmentation cutanée tels que les lentigos. Il est maintenant bien établi que le protéasome est responsable de la dégradation de la tyrosinase et un équilibre entre sa dégradation et la synthèse de cette enzyme est nécessaire pour une pigmentation régulière de la peau, des cheveux et des yeux chez l'homme. Dans une étude précédente (Bulteau et al., *Antioxidants and Redox Signaling*, 2006, **1-2**. 136-143) nous avions analysé les effets d'un extrait issu de l'algue unicellulaire *Phaeodactylum tricornutum*, sur les activités peptidasiques du protéasome de kératinocytes humains en culture et montré que cet extrait présentait un effet stimulateur et protecteur sur le protéasome. Nous décrivons ici l'effet activateur de cet extrait sur les activités peptidases du protéasome de mélanocytes humains. Cette stimulation de l'activité du protéasome résulte en une augmentation de la dégradation de la tyrosinase et par conséquent une réduction de l'activité enzymatique associée permettant ainsi de définir de nouvelles stratégies de traitement des cellules épidermiques contre les effets néfastes de l'accumulation de mélanine. Des expériences d'immunoprécipitation ont aussi montré qu'en présence de cet extrait la tyrosinase était moins ubiquitinée suggérant que cet extrait pourrait également jouer un rôle sur la machinerie d'ubiquitination.

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Neuroprotective effects of phenolics compounds from Mediterranean diet

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The Mediterranean diet (MeDi) has received increased attention in recent years because it is associated to lower risk for cardiovascular disease, several forms of cancer, and overall mortality. Recently, it has been observed that higher adherence to the MeDi is associated with a reduction in risk for Alzheimer's disease (AD).
The MeDi is characterized by high intake of vegetables, fruits, red wine and the consumption of a large amount of olive oil which is a source of unsaturated fatty acids and at least 30 phenolic compounds. The major phenolic compounds in olive oil are oleuropein, hydrotyrosol, tyrosol., cinnamic acid or coumaric acid. In human, their bioavailability has been observed.
We have tested the hypothesis that these phenolics compounds, that act as free radical scavengers, could interfere with oxidative stress caused by the amyloid-ß peptide, the main neurotoxin involved in the pathogenesis of AD. Moreover, we have investigated their protective effect against the Parkinsonian neurotoxin 1-methyl-4-phenyl-1,2,6-pyridinium (MPP^+). This protective effect was analyzed on cell culture models using PC12 and N2a cell lines. Our results demonstrate that these phenolics compounds could attenuate the decrease of the antioxidant glutathione and cell mortality induced by these neurotoxins. This protective effect is observed through different mechanisms, including the free radical scavenger but also through the regulation of the transcription factor NF-kB.

P56

Anti-aging and anti-carcinogenic effects of orally administered Pycnogenol® in mice treated with UVR and combination of DMBA-UVR

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Reactive oxygen species (ROS) can be frequently generated in biological systems either by normal metabolic pathways or as a consequence of exposure to physical and chemical agents. It is also known that ROS contribute to aging, mutagenesis, carcinogenesis and many diseases. Chemoprevention represents a relatively new and promising strategy which can slow, reverse or completely halt this process by the use of natural or synthetic antioxidants**.**
The pine bark extract Pycnogenol® obtained from the French Maritime Pine Pinus Maritima, owning to the chemical structure of its components, presents remarkable antioxidant activity. Although many strong antioxidant substances have been studied for their preventive-anticancer activity, no sufficient data exist yet for Pycnogenol®. The aim of this study was to examine the preventive activity of Pycnogenol® from the oxidative, aging and genotoxic effects of extrinsic environmental factors simulated by UVR and DMBA.
Pycnogenol® preventive activity was evaluated in two different experimental animal tumor models in which tumors were induced by ultraviolet radiation (UVR) and combination of UVR with 7,12-dimethylbenz[a]anthracene. The animals were examined weekly, for nearly a year, for the appearance of aging signs, pigmented lesions, papillomas, basal and squamous carcinomas. The effect of Pycnogenol® on experimental carcinogenesis was evaluated on the basis of comparing results of the above examination between test and control groups.
Significant decrease in the number of animals bearing tumors and in the number of tumors per animal was observed in the Pycnogenol® treated animals. Significant retardation in the appearance of tumors and increase in the viability of these animals was also observed. Furthermore, reduction of aging signs such as wrinkles, skin laxity and pigmented lesions was observed at Pycnogenol® treated animals before papillomas appearance. These results seem to be very promising for the protective activity of Pycnogenol® against extrinsic aging and carcinogenic factors such as UVR (to which the major part of the population is exposed) and chemical pollutants (to which particular population groups are exposed during their work).

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Olive Oil: A Functional Lipid Food

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Olive oil, a natural juice obtained from the fruit of the tree *Olea europaea*, is the main constituent of the Mediterranean diet. It is not only a flavorful lipid food but also a product with a unique composition. Olive oil is a naturally monounsaturated oil, rich in phenol and aroma compounds. Its composition and quality are affected by several factors such as cultivar, fruit maturity, micro-environment where the trees are grown, processing technology of olive fruit (two or three - phase centrifugation systems) and conditions applied during fruit processing such as, temperature, time etc. Phenol concentration is diminished with advanced fruit maturity, while aroma compounds are exists in higher concentration at the optimum fruit maturity stage.

Certain populations have traditionally enjoyed overall good health. Those living in the Mediterranean area of the world have been considered to be among the healthiest people. While a wide range of factors certainly contribute to this good health, available information suggests that specific components of the diet play a great role. High intake of monounsaturated fat, typically in the form of olive oil, has been a main constituent of this diet. Thee elevated LDL level and its oxidation in the body, is now believed to raise CHD risk. Olive oil, containg polyphenols and flavons, aroma and other important compounds is believed to help the functions of our body and protect it from different diseases such as CHD.

The purpose of this poster is to present the main constituents of olive oil, how they are affected by several factors and to provide a brief overview of evidence that olive oil has to be accepted as an important functional lipid food.

P58

Walnuts tropical oil decreases UV induced-DNA damage and Mélanoma Cell proliferation with UV filter Properties and P2X7 cell death receptor modulation

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UV radiations can induce reactive oxygen species formation, leading to various ocular diseases or skin cancer. Aging induces a decrease in the production of antioxidants and antioxidative enzymes with accumulation of endogenous molecules that are phototoxic. Because most UV filters are cytotoxic for eye, we investigated the anti-UV properties of walnuts tropical oil in order to propose it as a potential therapeutic additive in ocular diseases or skin cancer prevention. Walnuts tropical oil (Calophyllum), even at low concentration (1/10000-v/v), exhibited significant UV absorption properties (max at 300 nm) and was associated with an important Sun Protection Factor (18-22) . Low concentrations of the oil were not cytotoxic on human conjunctival epithelial cells line WKD. In hight concentrations, Walnuts tropical oil has anti-proliferative effect through P2X7 cell death receptor activation in melanoma cells line B16-F0. Walnuts tropical oil appeared to act as a cytoprotective agent against oxidative stress and DNA damage (*1) (85% of the DNA damage induced by UV radiations was inhibited with 1% Calophyllum oil) and did not induce *in vivo* ocular irritation (Draize test on New-Zealand rabbits). Walnuts tropical oil thus exhibited antioxidant and cytoprotective properties, and therefore might serve, for the first time, as a natural UV filter in ophthalmic preparations and therapeutic additive in skin cancer .
(*1)-T. Said et al., Eur. J. Pharm. Sci (2007) 30 : 203-210

P59

Alterations Of Proteins In Striatum Of Dependent Alcohol- Inhaled Rats. Role Of Oxidative Stress.

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Brain is very sensitive to the free radical attack and a target for the toxicity of ethanol via the oxidative stress. An increase in the CYP 2E1, supporting the production of free radicals, from oxygen and the radical hydroxyethyl was evidenced after chronic alcoholisation in striatum of rat. Oxidative damage of proteins due to the oxidative stress and the activities of the proteasome 20S, which eliminates abnormal proteins, were evaluated after 4 weeks of alcoholization per inhalation and at 24 hours of withdrawal in 6 Wistar adult male rats. These rats were physically and behaviourally dependent and 4 control rats were subjected to the same treatments but alcoholisation. Carbonyl proteins of striatum were measured after separation by electrophoresis on polyacrylamide gel and marking of the carbonyls groups by Western-blot using an anti-DNP antibody. The displayable bands were quantified. Two peptidasic activities of the proteasome 20S: chymotrypsin-like and peptidylglutamyl peptide hydrolase, were determined. Proteasomic activities were not significantly modified in any group of rats. The protein molecular weights of the protein carbonyls spread out of 43kD over 145kD. No significant modifications were found in the alcoholic rats vs. controls while the band located at about 54kD was significantly increased in the withdrawn rats (+19%, $p<0.05$). Other bands displayed a non-significative increase in carbonyl groups attesting of a preferential attack of certain proteins, to be determined especially because they could be of interest in alcoholism. The damage due to free radicals during ethanol withdrawal could be related to high glutamate release and symptoms as the hyperactivity. Furthermore, as the striatum is a brain area very implicated in the dependence development in brain, the toxicity expressed at withdrawal could participate to the setting up and persistence of ethanol dependence.

P60

Health improving effects of Sage tea – a pilot study

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Salvia officinalis (common sage) is a medicinal plant to which antioxidant, anti-inflammatory and antimutagenic properties have been attributed. Recent results from our lab also show that a water extract of *Salvia officinalis* reduces liver glucose production and fasting plasma glucose levels in rats, suggesting an antidiabetic potential for sage. In order to test these effects in humans we performed a pilot trial, with six healthy volunteers. The trial was carried out in three phases which include two weeks of baseline, four weeks of sage tea treatment and two weeks of wash out. Sage treatment positively affected the erythrocyte antioxidant status as shown by the increase of SOD and CAT activities. Cholesterol and LDL levels significantly decreased and HDL levels significantly increased after treatment, indicating benefits also in lipid metabolism. However there were no changes in response to oral glucose tolerance tests. Significant reduction of lymphocyte DNA damage induced, in vitro, by H_2O_2, was observed in association with significant increases of Hsp70 expression. Despite the fact that there were no effects on blood glucose levels, all the other results confirm the health improving potential of sage tea drinking, including for diabetic patients.

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P61

Effects of gold compounds, after selenite treatment, in cisplatin-sensitive and-resistant ovarian cancer cells

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The development of drug resistance is a major cause for the failure of chemotherapy. In the present work we have studied the effects of auranofin, a gold(I) compound, on cisplatin sensitive (2008) and resistant (C13*) human ovarian cancer cells. Auranofin is more effective than cisplatin in decreasing cell viability and its effect is more marked in cisplatin-resistant than in sensitive cells. Furthermore, auranofin is able to permeate C13* cells more efficiently than 2008, determining a consistent release of cytochrome c both in resistant and in sensitive cells. Treatment with auranofin determines an increased production of reactive oxygen species that is larger in C13* cells compared to 2008. However, H_2O_2 production is counteracted in C13* cells by a large overexpression of thioredoxin reductase, while glutathione reductase is slightly incremented. However, the resistant phenotype, differently from the sensitive one, is characterized by very high levels of glutathione.
Selenite is able to act as a substrate of thioredoxin reductases and to interact with glutathione. In addition, sodium selenite, in isolated mitochondria, determines membrane permeability transition resulting from a thiol redox shift. We have further observed that, sodium selenite, in the nanomolar range, increases cell viability in both cell lines, while at higher concentrations decreases cell growth. Interestingly, cell pre-treatment with selenite dramatically increases the cytotoxic effect induced by auranofin in resistant, but not in sensitive cells.

P62

Flavonoids and their oxidation products protect efficiently albumin-bound linoleic acid in a model of plasma oxidation

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Although LDL esterified polyunsaturated fatty acids (PUFA) contribute largely to the pool of oxidizable lipids in plasma, they coexist with a non-negligible content of free PUFA. In some pathological conditions such as diabetes, the free PUFA/albumin ratio becomes abnormally elevated. Modeling was performed in a system constituted of linoleic acid bound to human serum albumin (HSA) in which oxidation was initiated by hydrophilic AAPH peroxyl radicals. Albumin has also been shown to be the carrier of the bioavailable forms of dietary flavonoids.[1] The aim of the present work was to investigate the antioxidant potential of various flavonoids in these plasma-mimicking conditions. The peroxidation of linoleic acid bound to human serum albumin (LA/HSA ratio = 4) led to the formation of isomeric hydroperoxyoctadecadienoic acids (HPODE), ketooctadecadienoic acids (KODE) and hydroxyoctadecadienoic acids (HODE), the latter resulting from an apparent peroxidase activity of albumin.[2,3] The accumulation of lipid oxidation products was inhibited by flavonoids and models of plasma metabolites as follows: isoquercitrin > quercetin > catechin = isorhamnetin >> kaempferol > quercetin-4'-β-D-glucoside = quercetin-3,4'-di-β-D-glucoside.[3] Surprisingly, quercetin and isorhamnetin afforded a protection to linoleic acid long after their consumption. Elucidation by MS and NMR of the quercetin oxidation products and assessment of their antioxidant capacity pointed out that 3,4-dihydroxybenzoic acid and 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2*H*)-one are major contributors to the apparent quercetin antioxidant capacity. In conclusion, albumin-bound flavonoids can be fully regarded as antioxidant species in plasma.

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