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OXIDANTS AND ANTIOXIDANTS IN BIOLOGY

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BOOK OF ABSTRACTS

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OXIDANTS AND ANTIOXIDANTS IN BIOLOGY

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CONTENTS

KEYNOTE ADDRESS

Thioredoxin and glutaredoxin systems: From basic science to clinical applications

Arne Holmgren

Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-171 77 Stockholm, Sweden

Thioredoxin (Trx) and thioredoxin reductase (TrxR) were originally purified as an NADPH- dependent hydrogen donor for *E.coli* ribonucleotide reductase (RNR). The active site sequence of Trx with the two redoxactive cysteine residues (CGPC) and its three-dimensional structure (thioredoxin fold) today defines a large family of proteins. The discovery that Trx and TrxR was identical to protein disulfide reductase has generated a large and ever growing list of functions for thioredoxin systems such as control of photosynthetic enzyme regulation, control of transcription factor activity, defence against oxidative stress or apoptosis or thiol redox control of cellular function, which will be discussed. Secretion of human Trx revealed functions for Trx and as a cocytokine and chemokine or for truncated Trx (Trx80) as a Th1 immune modulator. The large TrxR from mammalian cells with broad substrate specificity including selenium compounds are selenoenzymes. Clinically used drugs targeting the highly reactive selenocysteine residue in the active site mammalian TrxR or RNR of tumor cells will be described.

Glutaredoxin (Grx) was discovered in *E.coli* mutants lackingTrx1 as another NADPH-dependent hydrogen donor system dependent on glutathione (GSH) and glutathione reductase Recent results regarding the role of Grx1 as an electron donor for mammalian RNR will be discussed. The mitochondrial human isoform, Grx2a, has recently been isolated as an iron-sulfur protein and shown to play a major role in defence against apoptosis via the mitochondrial pathway.

SESSION I
OBESITY, THE METABOLIC SYNDROME, AND OXIDATIVE STRESS

Role of oxidative stress in diseases associated with overweight and obesity

Jason D. Morrow

Vanderbilt University School of Medicine, Nashville, TN, USA

Overweight and obesity are major public health problems in the United States and predispose to important diseases such as atherosclerosis. We and others have determined that oxidant stress as quantified by F2-isoprostane (IsoP) formation is significantly increased in overweight persons and those with the metabolic syndrome. IsoPs are generally regarded as the most accurate measure of oxidant stress in vivo. Recent studies suggest that obesity-associated oxidant stress can be diminished in humans with weight loss and/or caloric restriction. These data will be discussed. A subclass of obesity is the metabolic syndrome. Cardiovascular disease in these individuals can be prevented by treatment with fish oil that contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Data are presented showing that EPA and DHA supplementation of humans and animals markedly reduces the formation of F2-IsoPs derived from arachidonate and increases the generation of EPA-derived and DHA-derived IsoPs. F2-IsoPs are generally regarded as proinflammatory and thus one beneficial effect of fish oil may be to decrease proinflammatory F2-IsoP production. We have also identified a class of reactive EPA-derived IsoPs termed cyclopentenone IsoPs that possess potent antioxidant properties by stabilizing the transcription factor Nrf2, increasing Nrf2 gene expression and lowering indicators of oxidant stress in cells and murine cardiac tissue. Together, these data suggest possible mechanisms for an anti-inflammatory and antioxidant role for EPA and DHA in the treatment of obesity-associated cardiovascular disease.

Macronutrient intake induces oxidative and inflammatory stress while insulin causes suppression of reactive oxygen species generation and inflamamtion

Paresh Dandona

Kaleida Health, Buffalo, NY, USA

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

Impact of weight reduction in diabetes type 2 on inflammation and oxidative stress

David Heber

UCLA Center for Human Nutrition, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Adipose tissue is an endocrine organ regulating whole-body metabolism, inflammation and immune responses. Adipocytokines identified to date are leptin, adipsin, resistin and adiponectin TNF-α, IL-6 and IL-10, which are thought to adapt metabolic fluxes to the amount of stored energy. Deregulation of this network secondary to central/abdominal obesity has been implicated in the etiology of insulin resistance, glucose intolerance, dyslipidaemia and increased blood pressure. As these are all risk factors for coronary heart disease, there is a major effort underway to define the metabolic problems related to obesity and find practical solutions. Reduction of excess adipose tissue especially intra-abdominal fat through the use of meal replacements, healthy diets, exercise and behavior change can result in significant reduction in inflammatory markers with only modest weight reductions on the order of five percent of body weight. The latter hypothesis is strengthened by our data showing that a reduction in abdominal fat of only 5 percent in type 2 diabetes patients leads to a reduction in C-reactive protein of ca. 30 percent. We have conducted cross-sectional studies of obese patients with and without metabolic syndrome and demonstrated that insulin resistance is the major factor distinguishing groups of patients with equivalent amounts of abdominal fat. This suggests that additional genetic factors may be involved in the metabolic syndrome. Nutrigenomic approaches are needed to optimize obesity treatment in the future by defining those patients at risk and the genetic/molecular targets involved.

Nutritional modulation of inflammation in the metabolic syndrome

Ishwarlal Jialal

Laboratory of Atherosclerosis and Metabolic Research, Department of Pathology, UC Davis Medical Center, Sacramento, CA, USA

Metabolic syndrome (Mets) is a disorder comprising of adiposity, dyslipidemia, abnormal glucose tolerance, and hypertension. It confers an increased risk for diabetes and cardiovascular disease (CVD). Inflammation plays a pivotal role in atherosclerosis and is involved in abnormalities associated with MetS such as insulin resistance (IR) and adiposity. The various biomarkers of inflammation, such as inflammatory cytokines (TNF-α, IL-6, and IL-1β), chemokines (MCP-1 and IL-8) as well as C-reactive protein (CRP) are increased in obesity and correlate with IR and CVD. IR is also associated with endothelial dysfunction (ED). Inflammation, IR, and ED amplify the cascade of metabolic and vascular derangements. The etiology of this syndrome is largely unknown but presumably represents a complex interaction between genetic metabolic, and environmental factors including diet. Since MetS is associated with chronic low-grade inflammation, strategies are being explored to ameliorate the pro-inflammatory status and MetS has been identified as a target for diet therapy to reduce risk of CVD. Weight loss appears to be the best modality to reduce inflammation. Intervention trials convincingly demonstrate that weight loss reduces biomarkers of inflammation, such as CRP and IL-6. The main treatment option for MetS includes life style changes, such as diet, exercise, and weight reduction. Majority of the studies have shown that therapeutic lifestyle changes (TLC), particularly weight loss, result in a decrease in biomarkers of inflammation and improvement in IR in MetS subjects. A randomized controlled trial of a Mediterranean-style diet versus prudent diet in patients with MetS resulted in not only significant reduction in the body weight, but also significantly reduced CRP, IL-6 as well as insulin resistance. Also, the anti-inflammatory effects of a dietary portfolio treatment have been postulated in hyperlipidemic adults with regards to the reduction of CRP levels. Furthermore, exercise has been shown to reduce chemokines (MCP-1 and IL-8) in subjects with MetS. Thus, TLC is strongly suggested for MetS subjects not only for weight reduction but also to reduce inflammation associated with this syndrome. Much further research is needed to define the role of individual dietary factors on the biomarkers of inflammation and the mechanism of the anti-inflammatory effects of weight loss in metabolic syndrome.

Oxidative stress in the adipocyte: Local and systemic consequences

Philipp E. Scherer

Departments of Cell Biology and Medicine
Albert Einstein College of Medicine, Bronx, NY 10461

Until recently, adipose tissue has been considered to be a mere storage compartment of triglycerides. It is now clear that adipocytes are highly active endocrine cells that play a central role in overall energy homeostasis and are important contributors to some aspects of the immune system. They do so by influencing systemic lipid homeostasis, but also through the production and release of a host of adipocyte-specific and adipocyte-enriched hormonal factors, cytokines and extracellular matrix components (commonly referred to as "adipokines"). Adipocytes fail to downregulate glucose uptake in response to hyperglycemic conditions. As a result, hyperglycemia induces high levels of ROS in adipocytes. This induces a local pro-inflammatory response in the adipocyte. Due to the paracrine interactions between the adipocyte and the local macrophages, the hyperglycemia-induced pro-inflammatory response in adipose tissue translates into a systemic elevation of inflammation.

In addition, the extremely long half-life of the adipocyte renders this cell type particularly prone to long-term effects of high ROS at the level of DNA damage. The highly active secretory pathway of the adipocyte is also susceptible to small changes in the redox potential induced by ROS-mediated changes in glutathione levels. The formation and disruption of critical disulfide bonds within the quaternary structure of adipokines has a major impact on the bioactivity of these major secretory proteins, such as adiponectin and resistin and may contribute towards an increased stress response within the secretory pathway under hyperglycemic conditions.

In summary, local ROS-induced effects in adipose tissue translate into systemic changes at the level of inflammation and through altered bioactivity of critical adipokines may mediate significant changes at the level of metabolism.

A new model for type 2 diabetes:progressive defect in β-cell mass in rats transgenic for human islet amyloid polypeptide

Peter C. Butler

Department of Medicine and Endocrinology, David Geffen School of Medicine, University of California, Los Angeles, California, USA

Type 2 diabetes (T2DM) is characterized by defects in insulin secretion and action, and is preceded by impaired fasting glucose (IFG). The islet anatomy in IFG and T2DM reveals an ~50% and 65% deficit in β-cell mass, with increased β-cell apoptosis, and islet amyloid derived from islet amyloid polypeptide (IAPP). Defects in insulin action include both hepatic and extrahepatic insulin resistance. The relationship between changes in β-cell mass, β-cell function and insulin action leading to T2DM are unresolved, in part because it is not possible to measure β-cell mass in vivo, and most available animal models do not recapitulate the islet pathology in T2DM . We evaluated the HIP rat, a human IAPP transgenic rat model that develops islet pathology comparable to humans with T2DM, at 2 age 2 months (non diabetic), 5 months (with IFG) and 10 months (with diabetes) to prospectively examine the relationship between changes in islet morphology versus insulin secretion and action. We report that increased β-cell apoptosis and impaired first phase insulin secretion precede development of IFG which coincides with an ~50% defect in β-cell mass and onset of hepatic insulin resistance. Diabetes was characterized by ~ 70% deficit in β-cell mass, progressive hepatic and extra-hepatic insulin resistance, and hyperglucagonemia. We conclude that IAPP induced β-cell apoptosis causes defects in insulin secretion and β-cell mass that lead first to hepatic insulin resistance and impaired fasting glucose, and then to extrahepatic insulin resistance, hyperglucagonemia and diabetes. We conclude that a specific β-cell defect can recapitulate the metabolic phenotype of T2DM, and note that insulin resistance in T2DM may at least in part be secondary to β-cell failure.

SESSION II
OBESITY, UNCOUPLING PROTEINS, AND MICRONUTRIENT ACTION

Obesity is associated with dysregulation of metabolic and inflammatory pathways in pregnant women

J.C. King[1], R. Mukherjea[1], L. Kunz[2], M. Millet[1], D. El Kady[3], J. DeHaene[2], P.J. Havel[2], K.L. Stanhope[2], J.L. Graham[2], and L. Woodhouse[4]

[1]*Children's Hospital Oakland Research Institute, Oakland, CA*
[2]*University of California, Davis, CA*
[3]*North Shore University Hospital, Manhassett, NY*
[4]*Western Human Nutrition Research Center, Davis, CA*

The prevalence of obesity among pregnant women is at an all-time high. This has implications for morbidity and mortality in both mother and baby. The risk of pregnancy-induced hypertension and metabolic complications, such as gestational diabetes, is significantly greater if the mother is obese. Growing evidence suggests there is a link between obesity, inflammation, and insulin resistance in non-pregnant and pregnant individuals. Adipokines secreted by the placenta, such as leptin and TNF-α, are thought to mediate that link in pregnancy. We recently evaluated the association between body mass index (BMI), insulin resistance, the inflammatory marker C-reactive protein (CRP), and the adipokines leptin and TNF-α. At 28 weeks gestation, maternal BMI was significantly correlated with insulin concentrations ($p<0.001$), HOMA, an indicator of insulin resistance, ($p<0.001$), leptin ($p<0.001$), C-Reactive Protein ($p<0.001$), and TNF-α ($p<0.005$). These findings suggest that placental activation of inflammatory pathways induce the maternal insulin resistance required for fetal growth in late pregnancy, but that the response is exacerbated by obesity and increases the risk of gestational diabetes. Potential treatments or interventions to ameliorate an enhanced inflammatory response in obese women are unknown.

Obesity due to metabolic malprogramming in early life

Mulchand S. Patel and Malathi Srinivasan

State University of New York at Buffalo, Buffalo, NY 14214

The obesity epidemic observed in western societies in recent years is a cause of concern since obesity is a risk factor for adult-onset metabolic diseases. An altered nutritional experience in early periods of life is one of the contributing factors in the etiology of obesity. We have investigated the long-term consequences of a high carbohydrate (HC) dietary modification (in contrast to high fat rat milk) during the suckling period in rats. Such a treatment results in immediate adaptations in pancreatic islets (resulting in the immediate onset of hyperinsulinemia) and hypothalamus (alterations in energy circuitry predisposing for hyperphagia). These early adaptations persist into adulthood despite weaning onto laboratory chow resulting in the HC phenotype of chronic hyperinsulinemia and adult-onset obesity. The HC dietary treatment in female rats results in a modified intrauterine environment (over weight, hyperinsulinemia and insulin resistance) during pregnancy. Fetal development in the HC female rat results in the spontaneous transfer of the HC phenotype to the progeny. In second generation (2-HC) rats hyperinsulinemia, altered insulin secretory response by fetal islets and alterations in the energy circuitry in the hypothalamus are evident in fetal life. These early changes persist into adulthood resulting in the onset of the HC phenotype in adult 2-HC rats. Our results suggest that alterations in feeding practices for babies (early introduction of cereals, fruits, etc.) and babies born to obese/hyperinsulinemic mothers may be contributing factors for the obesity epidemic.

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UCP2, oxidative stress and the pancreatic β-cell

J. Diao J and M.B. Wheeler

Department of Physiology, Faculty of Medicine, University of Toronto, Canada

Mitochondria have been considered a substantial source of reactive oxygen species (ROS). Activation of mitochondrial uncoupling, specifically by activation of uncoupling proteins (UCP) such as UCP2, may counterbalance the oxidative stress and attenuate cellular oxidative damage. For example, UCP2 can reduce ROS induced by inflammatory factors in brain cells and macrophages. However, an increase in mitochondrial uncoupling also raises the possibility that the cell may become more sensitive to apoptotic insults. UCP2 has been linked to type 2 diabetes and serves as an endogenous negative regulator of insulin secretion from pancreatic beta cells. However, its molecular function in pancreatic islets is still an area of great debate. We have sought to determine whether up-regulation or down-regulation of UCP2 in the beta cell can improve islet function. We established that UCP2 knockout mice (UCP2-/-) exhibited superior glucose sensing associated with an increased islet mass following a high fat diet. In addition, UCP2-/- islets *in vitro* maintained better insulin secretion than wild type (WT) islets in response to glucose after exposure to free fatty acids (FFA). To explore the mechanisms underlying these findings, we examined ROS levels, apoptosis and necrosis in islets (intact islet and dispersed islet cells) isolated from UCP2-/- and WT mice. The absence of UCP2 had no effect on the ROS production or apoptosis induced by strong ROS generators such as menadione, ceramide or cytokines in islets and specifically in beta cells. We also assessed the effect of UCP2 over-expression in the well-established pancreatic beta cell line, MIN6. Interestingly, UCP2 over-expression decreased total cellular ROS induced by FFA and enhanced cell viability. In addition, mitochondrial ROS formation was reduced in UCP2-overexpressing MIN6 cells, although UCP2 offered no protection from menadione- or cytokine-induced apoptosis. We observed that endogenous UCP2 protein in MIN6 cells could be induced by many factors including FFA and cytokines. Whether this serves as a defense mechanism against cellular stress or is a consequence of cell stress, is yet unknown. Our data suggest that expression of UCP2 in pancreatic cells *in vitro* may contribute to counterbalance the mild oxidative stress. Conversely, under chronic lipotoxic stress *in vivo*, the absence of UCP2 has beneficial effects on insulin secretion from pancreatic beta cell.

Modulating mitochondrial function in adipocytes and pancreatic β-cells with mitochondrial nutrients: Effects on UCP2, insulin, and oxidative stress

Jiankang Liu

Children's Hospital Oakland Research Institute and University of California at Berkeley

We propose that preventing and/or ameliorating oxidative mitochondrial dysfunction may have preventive and therapeutic potential. In the present study, we first investigated the protective effects of mitochondrial nutrient R-alpha-lipoic acid (LA) and acetyl-L-carnitine (ALC) on chronic oleic acid -induced pancreatic beta-cell dysfunctions. Long-term exposure of oleic acid to MIN6 cells caused a suppression of glucose-stimulated insulin secretion. This suppression is accompanied by an increase in intracellular oxidant formation, a decrease in mitochondrial membrane potential, an enhancement of UCP-2 protein expression and decreased glucose induced ATP production. Pretreatment with LA or/and ALC could reduce oxidant formation, improve mitochondrial membrane potential, regulate UCP-2 mRNA and protein expression, and increase glucose induced ATP production, and ultimately, restore the glucose stimulated insulin secretion. We then investigated the effects of LA and/or ALC on mitochondrial biogenesis in 3T3-L1 adipocytes. LA or/and ALC treatment significantly increased mitochondrial mass, expression of mitochondrial electron transport proteins and expression of factors involved in mitochondrial biogenesis, including peroxisome proliferator–activated receptor-gamma coactivator-1 alpha, mitochondrial transcription factor A, NRF1 and NRF2. The combination of LA and ALC exhibited a strong synergistic effect at the lower doses, approximately, more than 10 fold effective than either LA or ALC alone. These results suggest that LA and ALC, especially, their combination, are effective on promoting mitochondrial biogenesis and thus, may be used for preventing and treating insulin resistance, type 2 diabetes, and obesity.

AMP-kinase as a potential target for anti-obesity therapy with dietary factors

Ock Jin Park

Hannam University, Daejeon, South Korea

AMP-activated protein kinase (AMPK) is activated during ATP-depleting metablic states such as nutrient starvation, vigorous exercise, ischemia-hypoxia. Increases in AMP/ATP ratio, or creatine/phosphocreatine ratio are known to activate AMPK via allosteric activation of AMPK by AMP or by phosphorylation of AMPK by AMPKK. The activated AMPK switches on ATP-generating pathways such as fatty acid oxidation to preserve the levels of ATP. As a highly conserved heterotrimeric kinase that functions as a major metabolic switch, AMPK emerges as a possible target molecule of anti-obesity. Dietary factors such as green tea catechins have been proposed as a chemopreventive for obesity, diabetes, cardiovascular diseases, etc. In the present study the molecular basis of dietary factors to control obesity via modulation of AMPK was investigated. In 3T3-L1 preadipocyte cells, EGCG, genistein, selenate and the other plant extracts strongly activated AMPkinase, prevented clonal expansion of pre-adipocytes and inhibited lipid accumulation. The inhibition of AMPK by a pharmacological approach dramatically induced adipogenesis, whereas the AMPK activator (AICAR) blocked adipocyte differentiation. The natural AMPK activators also stimulated PPAR-γ, a molecule plays a major role in adipogenesis through binding to specific ligands. Also, we tested the possible link between AMPK and PPAR-γ in adipocytes and cancer cells to provide the molecular basis of AMPK and PPAR-γ involvement of obesity or cancer. AICAR elevated PPAR-γ in adipocytes, whereas diminished PPAR-γ activity in cancer cells. It was hypothesized that modulation of energy balance in adipocytes is achieved by manipulating AMP-kinase with phytochemicals and AMPK as a novel target to block adipogenesis and a critical component of PPAR-γ activity.

Analysis of *Bcmo1* knock-out mouse model uncovers a role of β-carotene for the regulation of lipid metabolism

Johannes von Lintig*, Susanne Hessel*, Anne Eichinger[#], Andrea Isken*, Vitus Oberhauser*, Klaus Vogt*, Regina Goralczyk[#] and Adrian Wyss[#]

**University of Freiburg, Institute of Biology I, Animal Physiology and Neurobiology, Hauptstrasse 1, D-79104 Freiburg, Germany;*
[#]DSM Nutritional Products Ltd., R&D Human Nutrition and Health, P.O. Box 3255, CH-4002 Basel, Switzerland

Dietary lipids are not only nutritionally important, but serve as precursors for ligands that bind to nuclear and membrane receptors which influence many aspects in the mammalian life cycle. As a classic example, retinoids (vitamin A and its derivatives) are critical for processes ranging from development to vision, cell differentiation, and metabolic control. All naturally occurring retinoids (C_{20}) derive from dietary carotenoids (C_{40}) with pro-vitamin A activity. Recently, we molecularly identified a β,β-carotene-15,15'-monooxygenase (Bcmo1), which converts β-carotene to retinal. To address the *in vivo* role of Bcmo1 in mammals, we established a knock-out mouse model. On a diet supplemented with retinoids, homozygous progeny of this mouse strain were viable and fertile. When β-carotene was provided to these mice as the sole dietary source for vitamin A, they became vitamin A-deficient and accumulated the provitamin in large quantities. Additionally, we found that *Bcmo1*$^{-/-}$ mice had an increased body fat mass and showed liver steatosis, associated with elevated serum free fatty acid levels. Together, our analysis identifies Bcmo1 as the key enzyme in vitamin A synthesis, but also reveals a heretofore neglected link between β-carotene conversion to vitamin A and the control of lipid homeostasis in mammals.

Selenium as an anti-inflammatory agent: Mechanisms for its hepatoprotective effect

Oren Tirosh and Shani Shilo

School of Nutrition, The Hebrew University of Jerusalem, Rehovot, Israel

By the year 2025, it is estimated that more than 25 million people in the USA alone will suffer from liver damage related to the increasing prevalence of type II diabetes and obesity. TG and free fatty acid (FFA) accumulation in the liver is associated with NASH, characterized by an inflammatory response with evidence of hepatocyte damage and fibrosis that can progress to cirrhosis. Selenium (Se) is recognized as essential in animal and human nutrition and was suggested to be an efficient hepatoprotective agent. We sought to investigate in rats the effect of Se supplementation, on the mitochondrial protein MnSOD during naïve and inflammatory conditions. We found that Se enrichment increased MnSOD levels in total liver due to an increase in MnSOD transcription in liver Hepatocytes. Lipopolysaccharides (LPS) injection furtherer elevated MnSOD levels both in hepatocytes and Kupffer cells (KC). This was blocked in the sodium selenite (sSe) supplemented animals by specific inhibition of transcription of the gene in the rat liver KC. Furthermore, sSe supplementation blocked the KC IL-6 transcription in LPS treated animals, indicating a reduced inflammatory activity of liver macrophages that could explain the anti-inflammatory activity of Se demonstrated by the lower levels of blood ALT and IL-6 compared to LPS treated animals. It is therefore concluded that sSe supplementation, regulates differentially the transcription of MnSOD in rat liver Hepatocytes and KC, while elevating it in the former and decreasing MnSOD levels in the latter. In addition, Se abrogated the IL-6 transcription in KC in LPS injected animals. This new mechanism may explain the liver hepatoprotective effect of selenium.

SESSION III
DIETARY MODULATION OF CELL SIGNALING PATHWAYS

Modulation of cell signal transduction by chemopreventive phyto-chemicals

Zigang Dong

The Hormel Institute, University of Minnesota, Austin, MN 55912 USA

Consumption of plant-derived food, especially fruits and vegetables, has been linked to decreased risk of cancer. Laboratory studies with animals and cells in culture have shown cancer preventive activity of phytonutrients isolated from soy, tea, rice, noni, and many green, yellow, and orange fruits and vegetables. We have used cell culture, transgenic mice, and knockout mice models to examine the anti-cancer effects of these dietary factors at the molecular level. We have found that: (1) (-)-Epigallocatechin gallate (EGCG), the major active polyphenol in green tea, and theaflavins, the major active components in black tea, inhibit epidermal growth factor (EGF)- or 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced AP-1 and NFκB-dependent transcriptional activation. Further, we found that the anti-cancer activity by EGCG may be through binding with its "receptor" or high affinity binding protein. We have isolated and identified a few of EGCG target proteins. (2) Active compound from rice and other grains, inhibited TPA- or EGF-induced transformation and signal transduction through its effects on PI_3 kinase. (3) Resveratrol inhibited cell transformation through the induction of apoptosis, mediated through p53 pathways. (4) Phenethyl isothiocyante (PEITC) inhibited cell transformation, correlated with the induction of apoptosis. An elevation of p53 is required for PEITC-induced apoptosis. (5) Chemicals in ginger and hot pepper showed inhibition of AP-1 and cell transformation and apoptotic pathways in cells. Our studies indicated that the chemopreventive effect of these phytonutrients may target different signal transduction pathways.

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Regulation of inflammation and glucocorticoid signaling by dietary polyphenols

Irfan Rahman

Department of Environmental Medicine, University of Rochester Medical Center, Rochester, NY, USA

Reactive oxygen species (ROS) play a key role in enhancing the inflammation through the activation NF-κB and AP-1 transcription factors, and nuclear histone acetylation and deacetylation (chromatin remodeling) in various inflammatory diseases. We have recently shown that oxidative stress enhances lung inflammation via expression of pro-inflammatory mediators through inhibition of histone deacetylase activity and NF-κB transactivation in monocytes/macrophages and epithelial cells. We also show the antioxidant and/or anti-inflammatory effects of dietary polyphenols (curcumin-diferuloylmethane, a principal component of turmeric) and resveratrol (a flavanoid found in red wine), in the control of NF-κB activation, upregulation of glutathione biosynthesis gene via Nrf2 activation, scavenging effect of ROS by inducing glutathione peroxidase activity, chromatin remodeling and subsequently inflammatory gene regulation in macrophages and lung epithelial cells. We further demonstrate that these dietary polyphenols restore glucocorticoid functions (anti-inflammatory property) in response to oxidative stress by upregulation of histone deacetylase activity. The anti-inflammatory property of curcumin and resveratrol may be due to its ability to induce histone deacetylase activity by reversing the post-translationally modified proteins. These data provide new information on regulation of inflammatory response by dietary polyphenols at the molecular level and possibly a way forward to inhibit chronic inflammatory response in various diseases.

Flavonoid regulation of the cell cycle via interactions within MAP kinase and PI3 kinase/Akt signaling cascades

Jeremy P.E. Spencer, Dominique T. Nguyen, and David Vauzour

University of Reading, UK

Studies have suggested that diets rich in polyphenols such as flavonoids may lead to a reduced risk of cancer incidence. Our studies have investigated the potential of flavonoids and metabolite forms to inhibit cancer cell proliferation via specific interactions within cell signalling pathways. Dimer B2 but not epicatechin monomer inhibited the proliferation of, and triggered apoptosis in, Caco-2 cells. The dinitroso-derivative of dimer B2 (formed in the stomach from reaction with nitrous acid), and to a lesser extent dinitroso-epicatechin, also induced significant toxic effects in Caco-2 cells. Inhibitory effects on cellular proliferation were paralleled by early inhibition of ERK1/2 phosphorylation and later reductions in cyclin D1 levels, indicating modulation of cell cycle regulation in Caco-2 cells. We also investigated the intracellular metabolism of genistein in T47D tumorigenic and MCF-10A non-tumorigenic cells and assessed the cellular actions of resultant metabolites. Genistein selectively induced growth arrest and G2-M phase cell cycle block in T47D but not MCF10A breast epithelial cells. These anti-proliferative effects were paralleled by significant differences in the association of genistein to cells and in particular its intracellular metabolism. Genistein was selectively taken up into T47D cells and was subject to metabolism by CYP450 enzymes leading to the formation of both 5,7,3',4'-tetrahydroxyisoflavone (THIF) and two glutathionyl conjugates of THIF. THIF inhibited *cdc2* activation via the phosphorylation of p38 MAP kinase suggesting that this species may mediate genistein's cellular actions. THIF exposure activated p38 and caused subsequent inhibition of cyclin B1 (Ser 147) and *cdc2* (Thr 161) phosphorylation, two events critical for the correct functioning of the *cdc2*-cyclin B1 complex.

Modulation of Nrf2, MAPK, IKK by isothiocyanates and phenolic compounds from *in vitro* studies to *in vivo* pharmacological effects

Ah-Ng Tony Kong

Rutgers University, Piscataway, NJ, USA

Evolutionarily, animals had been ingesting plants. This "animal-plant" warfare has resulted in an elaborated system of detoxification and defense mechanisms evolved by animals including humans. Animal cells respond to these dietary phytochemicals by "sensing" these chemical-stress typified by 'thiol modulated" cellular signaling events leading to gene expression of either pharmacologically beneficial effects, but some time also unwanted cytotoxicity. Our laboratory has been studying two groups of dietary cancer chemopreventive compounds (isothiocyanates and polyphenols), which are effective against chemical-induced as well as genetically induced animal carcinogenesis models. These compounds typically generate "cellular stress" and modulate gene expression including phase II detoxifying/antioxidant enzymes GST, QR, HO-1 and GCS via the Keap1-Nrf2/ARE pathway. However, using Nrf2 -/- mice coupled with Affymetrix microarray analyses, other category of genes such as ubiquitination, electron transport, transporters, cell growth and apoptosis, cell adhesion, kinase and phosphotases and transcription factors modulated by these compounds appeared to be Nrf2-dependent, at least in normal tissues, leading to the overall cellular protective effects against oxidative stress or carcinogenic damages. Importantly, in tumor tissues, these compounds appear to simultaneously modulate differentially over-expressed growth signaling molecules such as the MAPK, IKK/NF-κB, Akt/mTOR signaling pathways culminating the apoptotic or autophagic cell death of tumor cells. The differential signaling/gene expression between normal versus "abnormal" cells would dictate the varied biological responses and pharmacological effects elicited by these dietary compounds.

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NF-κB and Nrf2 as prime chemopreventive and chemoprotective targets of anti-inflammatory and antioxidative phytonutrients and phytopharmaceuticals

Young-Joon Surh

National Research Laboratory of Molecular Carcinogenesis and Chemoprevention, College of Pharmacy, Seoul National University, Seoul 151-742, South Korea

There are multiple lines of compelling evidence supporting the association between inflammatory tissue damage and cancer. A new horizon in chemoprevention research is the recent discovery of molecular links between inflammation and cancer. Components of the cell signaling network, especially those converge on the ubiquitous eukaryotic redox-sensitive transcription factor, nuclear factor-kappaB (NF-κB), have been implicated in pathogenesis of many inflammation-associated disorders. A wide variety of chemopreventive and chemoprotective agents can alter or correct undesired cellular functions caused by abnormal pro-inflammatory signal transmission mediated by NF-κB. Modulation of cellular signaling involved in chronic inflammatory response by anti-inflammatory agents hence provides a rational and pragmatic strategy in molecular target-based chemoprevention and cytoprotection. Induction of phase-2 detoxifying or antioxidant genes represents an important cellular defensice in response to oxidative and electrophilic insults. Nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) plays a crucial role in regulating phase-2 detoxifying/antioxidant gene induction. Many antioxidants derived from dietary and medicinal plants have been found to activate this particular redox-sensitive transcription factor, thereby potentiating cellular antioxidant or detoxification capacity.

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Suppression of PhIP-induced tumors by white tea and EGCG, but promotion by caffeine

R. Wang, O. Carter, W.M. Dashwood, C. Lohr*, K. Fischer*, C. Pereira[+], R.H. Dashwood

*Linus Pauling Institute, *Dept Veterinary Medicine, [+]Dept Statistics, Oregon State University, Corvallis, OR97331*

Cooking of meat at high temperatures produces heterocyclic amines, including 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). We reported that white tea (WT) was more effective than green tea (GT) at preventing mutagenesis by PhIP in vitro. We now show that WT (but not GT) suppressed PhIP-induced colonic aberrant crypts in the rat, as did caffeine and epigallocatechin-3-gallate (EGCG) at concentrations equivalent to those in WT. In a 1-year tumor study, rats received PhIP followed by 2% WT, 0.05% EGCG, high-dose caffeine (0.08%, CAF-H), low-dose caffeine (0.05%, CAF-L), or drinking water. Compared with controls, rats given PhIP+CAF-H had shorter survival and a higher cumulative incidence of colon tumors, whereas EGCG improved survival and delayed the onset of tumors. Groups given PhIP+WT or PhIP+CAF-L had delayed onset and reduced incidence of Zymbal's gland tumors, lymphoma, and skin tumors, but a higher cumulative incidence of colon tumors, possibly due to better overall survival rates vs PhIP controls. β-Catenin/Tcf targets were highly expressed in colon tumors from all groups, but PhIP+CAF-H increased the frequency of codon 34 mutant β-catenin, and c-myc mRNA expression was elevated significantly ($P<0.05$ vs tumors with wild type β-catenin). In summary, protective effects were seen for white tea, EGCG, and low-dose caffeine, whereas high-dose caffeine promoted PhIP-induced colon tumors in the rat, possibly via selection of codon 34 mutant β-catenin and enhanced activation of c-myc.

Dietary modulation of oxidative stress and mucosal damage by *Helicobacter pylori* infection

Ki-Baik Hahm, Kee Myung Lee, Marie Yeo, Tae Young Oh, Misun Kwak, Sung Won Cho

Department of Gastroenterology, Ajou University School of Medicine

Debates that Helicobacter pylori (H. pylori) infection might play a causative role in gastric carcinogenesis still exists in spite of the World Health Organization's definition of H. pylori as a class I carcinogen. Though decreasing effects of carcinogenesis with the eradication of microbes were observed in animal models, no significant cancer prevention even after H. pylori eradication was proven in clinical trials, suggesting the modulation of predisposing gastric inflammations might be more important rather than simple eradication. Therefore, persistent inflammation and considerable levels of oxidative stress contributed to H. pylori-associated gastric carcinogenesis. iNOS-/- mice infected with H. pylori showed significantly attenuated incidence of gastric adenocarcinoma because the overproduction of NO via iNOS is suggested to be a significant pathogenic factor in H. pylori-induced gastritis, nimesulide, COX-2 inhibitor, prevented H. pylori-associated gastric carcinogenesis, restoration of heat shock protein 70 (Hsp 70) suppressed gastric mucosal iNOS expression, which is related to H. pylori-associated gastric carcinogenesis, and blockage of Hsp 90 modulated H. pylori-induced IL-8 productions through the inactivation of transcriptional factors of AP-1 and NF-κB. Another factor influencing the destinations of H. pylori infection related to malignancy might be the either host genetic background or environmental factor. Administration of α-tocopherols, green tea, and Korea red ginseng could decrease the inflammatory cascade and signal transduction, resulted in significant attenuation of H. pylori-gastric inflammation. A continuous presence of dietary factors and these dietary agents are able to reverse, suppress or prevent either the initiation or progression of H. pylori-associated gastric carcinogenesis.

Chemoprevention and inhibition of tumor metastasis by selenium compounds

An Sik Chung

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, 305-701, South Korea

Recent studies have implicated that cell cycle arrest and apoptosis are most feasible mechanisms of chemoprevention, and metalloproteases (MMPs) are a crucial factor involved in tumor invasion and metastasis. Our previous study demonstrated that Se-methylselenocysteine (MSC) induced apoptosis in HL-60 cells and that reactive oxygen species (ROS) played a crucial role in MSC-induced apoptosis. In these studies, it was shown that treatment with SeMet and METase decreased the expression of the integrins α4, α5, αν, β1, and β3, and inhibited melanoma-ECM-specific adhesion. Furthermore, G_1 cell cycle arrest and apoptosis were induced following loss of cell adherence. Phosphorylation of focal adhesion kinase (FAK) and Akt related to integrin-mediated survival were decreased by treatment with SeMet and METase while phosphorylation of p38, PKC-δ, and IκBα were increased. Using specific inhibitors against p38, PKC-δ, and NFκB, we demonstrated that the expression of integrins and adhesion to ECM were maintained, and induction of apoptosis was blocked in melanoma cells treated with SeMet. It was demonstrated that SeMet induced cell cycle arrest and apoptosis of melanoma cells, and these events were from the alteration of integrin expression and adhesion through activation of p38, PKC-δ, and NF-κB. Another study showed that low level of selenite (less than 3 μM) inhibited the invasion of tumor cells and also adhesion of HT1080 cells to the collagen matrix. Moreover, selenite reduced activity and expression of matrix mellaoproteinase-2, -9 and urokinase type plasminogen activator, which are involved in matrix degradation and tumor invasion, but increased those of a tissue inhibitor of metalloproteinase-1 (TIMP-1). This inhibitory effect of selenite on the proteases' expression was mediated by the suppression of transcription factor, NF-κB and AP-1. Further animal study was shown that administration of MSC or selenite to C57BL/6J mice increased survival rate and reduced tumor metastasis by injection of melanoma into the tail vein. 10 μM of SeMet (non-toxic low level) dramatically reduced tumor invasion in B16F10 cells, which was mediated by reduced activity and expression of MMP-2 and -9. These results suggest that high level of Se compounds are used for chemoprevention by induction of apoptosis and low level of Se compounds used for tumor invasion and metastasis by reduction of MMP-2 and -9 activity or expression.

Diets enriched in ω-3 fatty acids decrease amyloid burden and alter signal transduction in a mouse model of Alzheimer's disease

Greg M. Cole , Giselle P. Lim, Qiu-Lan Ma, Fusheng Yang, and Sally A. Frautschy

Greater Los Angeles Veterans Affairs Healthcare System, Geriatric Research, Education and Clinical Center, 16111 Plummer Street , Sepulveda, California 91343, Departments of Medicine and Neurology, University of California, Los Angeles, California 90095

Fish consumption and higher omega-3 (ω-3) fatty acid intake are associated with reduced risk for Alzheimer disease (AD). Because docosahexaenoic acid (DHA) is the major ω-3 in brain, we examined the impact of diets depleted of and enriched in DHA in an Alzheimer model, Tg2576 mice expressing a familial AD mutant form of the 695 amino acid human amyloid precursor protein that gives rise to the 40 or 42 amino acid Aß amyloid peptide species that accumulate in ß-amyloid plaque deposits in AD. Mice were raised to 17 months of age on mouse breeder chow and then placed on an ω-3 depleting, high safflower oil diet ("BAD diet") with or without 0.6% supplemented DHA until 22 months of age. DHA reduced the levels of total Aß, Aß42 peptide and amyloid plaques. The experiment was repeated using mice fed 1/2 safflower oil and 1/2 oleic acid ("half-Bad diet") with and without DHA. Again, DHA reduced amyloid accumlation. Analysis of signal transduction changes in mice fed DHA are consistent with the hypothesis of increased neurotrophic or insulin-like signaling through the PI3-K> Akt leading to reduced Aß production and increased Aß clearance, notably through insulin degrading enzyme (IDE). Oxidative damage and caspase activation were both reduced. In addtion to reducing Aß, DHA also appears to act at multiple sites to limit Aß oligomer-induced defects in the rac>PAK signaling pathway and synaptic function. These results argue that diets enriched in DHA or DHA supplements can suppress both amyloid peptide levels and signal transduction deficits induced by toxic oligomer amyloid species. Clinical trials to assess DHA as a protective approach to Alzheimer's are underway.

Effects of lycopene on carotenoid cleavage enzymes I and II expression in various tissues of F344 rats and PPAR and RAR gene regulation

John W. Erdman Jr., Manabu T. Nakamura, Xiaoming Gong, Lewis P. Rubin and Susan Zaripheh

Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL. and Department of Cell Biology, Cleveland Clinic Foundation, Lerner Research Institute, Cleveland, OH, USA

In vitro studies have suggested that lycopene (lyc) is an efficient substrate for carotenoid 9'10'-mono-oxygenase II (CMO2) but may inhibit carotenoid 15, 15'-mono-oxygenase I (CMO1) activity. The overall objective of this study was to determine the effects of lyc treatment on CMO1 and CMO2 expression and on peroxisomal proliferators activated receptor (PPAR) and retinoic acid receptor (RAR) gene regulation. Rats were fed lyc (0.25g lyc/kg diet) for different lengths of time (3-37 d). The rat CMO2 gene was cloned and found to be 92% and 82% homologous to the mouse and human CMO2 nucleotide sequence, respectively. The relative abundance of CMO1, CMO2 and PPARγ were differentially expressed in rat tissues. Lyc intake decreased CMO1 and PPARγ expression in the kidney and adrenal ($P<0.05$), while CMO2 expression was only reduced in the kidney. Lyc intake also decreased expression of fatty acid binding protein 3 (FABP3), a PPARγ target gene, in the kidney and adrenal ($P < 0.05$). The effect of lyc on PPAR and RAR gene regulation in human cell lines was also investigated. Pulmonary epithelial A549 cells treated with lyc resulted in a time- and dose- dependent decrease in PPAR activity but no change in RAR activity as measured by the luciferase assay. A549 cells co-transfected with PPARγ and PPRE-luc reporter showed a 13% decrease in reporter activity with a 1uM lyc treatment for 24h. No effect of lyc (1-10uM) on PPAR or RAR activities was observed in DU145 prostate cancer cells. These data suggest that lyc may play an important role in the modulation of β-carotene and lipid metabolism.

Acetyl-carnitine induces heme oxygenase in rat astrocytes and protects against oxidative stress: Role of the transcription factor Nrf2

V. Calabrese, A. Ravagna, C. Colombrita, E. Guagliano, M. Calvani, D. Allan Butterfield and A. M. Giuffrida Stella

Department of Chemistry, Univ. of Catania, Italy and Department of Chemistry, University of Kentucky, USA

Heat shock response contributes to establishing a cytoprotective state in a wide variety of human diseases. Among the various heat shock proteins, heme oxygenase I, has received considerable attention, as it has been recently demonstrated that heme oxygenase-1 induction, by generating the vasoactive molecule carbon monoxide and the potent antioxidant bilirubin, could represent a protective system potentially active against brain oxidative injury. Acetyl-L-carnitine is proposed as a therapeutic agent for several neurodegenerative disorders. Accordingly, in the present study we report that treatment of astrocytes with acetyl-L-carnitine induces heme oxygenase-1 in a dose- and time-dependent manner and that this effect was associated with up-regulation of heat shock protein 60 as well as high expression of the redox-sensitive transcription factor Nrf2 in the nuclear fraction of treated cells. In addition, we show that addition of acetyl-L-carnitine to astrocytes, prior to proinflammatory LPS- and INFγ-induced nitrosative stress, prevents changes in mitochondrial respiratory chain complex activity, protein nitrosation and antioxidant status induced by inflammatory cytokine insult. Given the broad cytoprotective properties of the heat shock response, molecules inducing this defense mechanism appear to be possible candidates for novel cytoprotective strategies. Particularly, manipulation of endogenous cellular defense mechanisms, via acetyl-L-carnitine may represent an innovative approach to therapeutic intervention in diseases causing tissue damage, such as neurodegeneration.

Astaxanthin reduces hyperglycemia-induced oxidative stress in glomerular cells

Yuji Naito, Katsura Mizushima, Satomi Akagiri, Osamu Handa, Hiroshi Ichikawa, JiroTakahashi, and ToshikazuYoshikawa

Medical Proteomics, Kyoto Prefectural University of Medicine, Kyoto, Japan, 602-8566 and Fuji Chemical ind., Nakanikawa, Toyama, Japan, 930-0397

Hyperglycemia increases the production of reactive oxygen species (ROS) from the mitochondrial electron transport chain in renal mesangial cells. Because several studies have postulated a role of antioxidant in the glomerular dysfunction seen in diabetic nephropathy, we evaluated the effect of astaxanthin on mitochondrial ROS production in cultured human mesangial cells (HMCs), and investigated the effects of astaxanthin on experimental model of diabetic nephropahy. We first confirmed that incubation of HMCs with 25 mM glucose significantly increased mitochondrial ROS production, NF-κB activation, and TGF-β expression, and that astaxanthin inhibited these changes induced by hyperglycemia. Secondary, we reported that chronic treatment with astaxanthin reduced glomerular oxidative stress as well as inhibited the increase of urinary albumin in diabetic db/db mice. Finally, we investigate the effect of astaxanthin on the expression of these genes using a high-density DNA microarray. Glomerular cells were obtained from the kidneys of mice by laser capture microdissection. By comparison between diabetic db/db and non-diabetic db/m mice, 779 probes (3.1%) were significantly affected, up-regulated 550 probes and down-regulated 229 probes, at least 1.5-fold in the diabetic mice. Ingenuity signal analysis for up-regulated probes determined the mitochondrial oxidative phosphorylation pathway as a most significantly affected caronical pathway, which are associated with complex I, III, and IV located on the mitochondrial inner membrane, and the expression level of them was decreased in mice treated with astaxanthin. In addition, the expression of many genes associated with oxidative stress, collagen synthesis, and transforming growth factor signaling was enhanced in diabetic mice, and this enhancement was partially decreased in the astaxanthin-treated mice. In conclusion, this genome-wide nutrigenomics approach provided insight into genes and genetic putative pathways that affected by the high-glucose stimulation as well as those involved in the anti-diabetic mechanism of astaxanthin.

Genepotency: A new approach to assess and compare the biological activities of natural compounds

Luca Barella[1], Elisabeth Stoecklin[1] and Gerald Rimbach[2]

[1]DSM Nutritional Products, Research & Development, CH-4002 Basel, Switzerland. [2]Instutute of Human Nutrition and Food Science, Christian-Albrechts-University, D-24118 Kiel, Germany

While vitamin E from natural sources occurs as a single stereoisomer (RRR-α-tocopherol), so-called synthetic vitamin E (all-rac-α-tocopherol) is an equimolar mixture of eight stereoisomers. In this study, we have used the Affymetrix GeneChip technology to evaluate the feasibility of a new bio-assay where the gene regulatory activities of RRR- and all-rac-α-tocopherol were quantified and compared on the genome-wide level. HepG2 cells were supplemented with increasing amounts of both tocopherols for 7 days. Genes showing a dose-related response were identified by global gene expression profiling. Our findings show that RRR- and all-rac-α-tocopherol share an identical transcriptional activity. Based on the transcriptional data, EC_{50} and IC_{50} values were determined for each of these genes. Subsequently, the calculation of a ′transcriptional potency factor′ was evaluated by dividing the EC_{50} / IC_{50} of RRR- by the corresponding EC_{50} / IC_{50} of all-rac-α-tocopherol for every vitamin E responsive transcripts. The mean of all potency ratios was found to be 1.05. In addition, clusters of genes sharing similar EC_{50} / IC_{50} values were identified. Such clusters were interpreted to contain genes controlled by promoters sharing similar/identical α-tocopherol responsive elements or transcription factors. In a next step, the promoter regions of genes grouping in the same cluster will be analyzed to possibly identify common, vitamin E, responsive structures.

KEYNOTE ADDRESS

Caspase-2 signaling in DNA damage-induced apoptosis

Sten Orrenius and Boris Zhivotovsky

Karolinska Institutet, Stockholm, Sweden

Caspase-2 is one of the best conserved caspases across species. Like other initiator caspases, it contains a pro-domain with a caspase-recruitment domain (CARD). Caspase-2 is engaged in the onset of apoptosis triggered by several insults, including DNA damage. Hence, caspase-2 is an important apical regulator in apoptotic pathways leading from DNA damage to release of cytochrome c and activation of effector caspases in 5-fluorouracil (5-FU)-treated colon carcinoma cells. Apoptosis was observed only in p53 +/+ cells and was preceded by caspase-2 activation. Proposed mediators of caspase-2 activation include the p53-inducible, death-domain containing protein, PIDD and the adaptor protein, RAIDD. Surprisingly, the presence of a complex involving RAIDD, PIDD and caspase-2 was verified in both p53+/+ and p53-/- cells, and caspase-2 activation was seen also in 5-FU-treated RAIDD- or PIDD-deficient cells. Thus, these results confirm the presence of PIDD and RAIDD in a PIDDosome complex with caspase-2, but question their role as sole mediators of caspase-2 activation. Our previous work has shown that processed caspase-2 can function as a link between DNA damage and activation of the mitochondrial pathway of apoptosis. Hence, processed caspase-2 can permeabilize mitochondria and trigger their release of cytochrome c and Smac/DIABLO, leading to activation of the caspase cascade. This effect is not seen with any other caspase, and it is independent of Bax/Bak and is not blocked by Bcl-2 overexpression. Neither does it require catalytically active caspase-2. It thus seems that processed caspase-2 can trigger the release of pro-apoptotic proteins from the mitochondria via pore formation, and that this release mechanism is equally efficient as Bax/Bak- or MPT-induced permeabilization of the mitochondria. The molecular basis for this unique property of caspase-2 is under investigation in our laboratory.

SESSION IV
MITOCHONDRIAL FUNCTION, AGING, AND DISEASE

Mitochondria: an end-point for nitric oxide signaling in ischemic pre-conditioning

Paul S. Brookes, Lindsay S. Burwell, Sergiy M. Nadtochiy

University of Rochester Medical Center, Rochester, NY 14642, USA

Ischemic preconditioning (IPC) is a phenomenon in which short non-lethal periods of ischemia and reperfusion (IR) can protect tissues such as the heart and brain from prolonged IR injury. Mitochondria are known to play a role in the mechanism of IPC, but the upstream signals are poorly understood. Similarly, nitric oxide ($NO^{\bullet}$) is known to be a critical regulator in IPC, but its downstream targets remain poorly characterized. Synthesizing these ideas with the knowledge that mitochondria are a major target for the signaling actions of $NO^{\bullet}$, it was hypothesized that $NO^{\bullet}$ mediated inhibition of the mitochondrial respiratory chain may be a cardioprotective mechanism in IPC. Reversible respiratory chain shutdown has previously been shown to protect the heart form IR injury, and this may be due to both inhibition of $\Delta\Psi$ driven Ca2+ uptake, and inhibition of ROS generation. In the current investigation we used proteomic techniques to identify several mitochondrial targets for $NO^{\bullet}$ mediated s-nitrosation, most notably the 75kDa subunit of complex I. In addition, we showed that mitochondrial s-nitrosation occurs during cardiac IPC. Furthermore, a series of mitochondrially targeted $NO^{\bullet}$ donors have been developed, and these compounds show potent cardioprotection in isolated cardiomyocyte and perfused heart models of IR injury. In support of a role for s-nitrosothiols in cardioprotection, a complex light dependence of recovery from cardiac IR injury was also observed. The upstream and downstream events surrounding $NO^{\bullet}$ mitochondrial interactions in IR injury and IPC will be discussed.

On the role of outer membrane channels in mitochondria-initiated apoptosis

Marco Colombini

University of Maryland College Park, Maryland U.S.A.

In addition to its many critical metabolic functions, mitochondria also have the ability to cause the entire cell to undergo programmed cell death, apoptosis. In many cell types, mitochondria somehow detect and integrate information about cellular metabolic state and decide whether to activate the cytosolic apoptotic system. While many mechanisms are under investigation, there is general agreement that the signal to the cytosol is the release of a set of proteins from the intermembrane space of mitochondria. In the cytosol these pro-apoptotic proteins initiate a cascade that leads to the execution phase of apoptosis. One event that leads to the release of these proteins is the closure of VDAC channels. VDAC normally facilitates the flow of virtually all metabolites between mitochondria and cytosol and thus VDAC closure signals to the mitochondrion a serious alteration in cellular state. This condition is reversible up to the point of protein release. VDAC closure occurs naturally upon removal of growth factors or can be induced by addition of specific artificial agents. Both result in protein release. The pathway for protein release is not VDAC. One pathway with the right properties is a ceramide channel. These highly-organized channels can form in the outer membrane as steady-state levels of ceramide rise but their formation may be inhibited or reversed by other factors, including other sphingolipids. Ceramide channels can form in phospholipid membranes in the total absence of proteins and can be visualized by electron microscopy. Bcl-2 family protein, well known for controlling the initial phase of the apoptotic process from the cytosolic side, can act on VDAC and may influence the formation of ceramide channels. Thus the regulation of VDAC gating and the formation of ceramide channels are likely to be critical to mitochondria-initiated apoptosis.

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Glutathione peroxidase 4 in oxidative stress-induced mitochondrial apoptosis and aging

Holly Van Remmen, Qitao Ran, Hanyu Liang, Wenbo Qi and Arlan Richardson

Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA

Lipid peroxidation is a major consequence of oxidative stress. Mitochondria are particularly vulnerable to lipid peroxidation, because of the high degree of poly-unsaturation in mitochondrial membrane lipids. Phospholipid hydroperoxide glutathione peroxidase (Gpx4) is a protective enzyme uniquely involved in the detoxification of oxidative damage to membrane lipids. Targeted deletion of Gpx4 is lethal in mice and Gpx4 deficiency in fibroblasts from heterozygous *Gpx4*$^{+/-}$ knockout mice results in increased vulnerability to oxidative injury. We propose that Gpx4 plays a critical role in protecting mitochondria from oxidative stress *in vivo* by reducing lipid peroxidation, and to study the role of Gpx4 in protecting mitochondria from oxidative stress, we have generated transgenic mice expressing Gpx4 (*Gpx4*Tg) mice. Cell death induced by oxidizing agents *t*-BuOOH and diquat is reduced in fibroblasts from *Gpx4*Tg mice compared to cells from wildtype mice. Diquat–induced liver damage (plasma ALT levels) and lipid peroxidation (plasma F_2-isoprostanes) are reduced significantly in *Gpx4*Tg mice compared to wildtype mice. Diquat induces inhibition of mitochondrial electron transport chain functions and reduced ATP production that is maintained in *Gpx4*Tg mice. In addition, diquat induced apoptosis through the mitochondrial apoptotic pathway (DNA fragmentation, cyt c release, and loss of mitochondrial membrane potential) is also attenuated in *Gpx4*Tg mice. These data demonstrate that Gpx4 plays a role *in vivo* in the mechanism of apoptosis induced by oxidative stress, likely through oxidative damage to mitochondrial phospholipids. Thus, Gpx4 transgenic and knockout mice are a potentially unique model for studying the role of oxidative damage in aging and age-related pathological processes.

Focused proteomics: towards a high throughput mAb based resolution of proteins for diagnosis of mitochondrial diseases

R.A. Capaldi

Institute of Molecular Biology, University of Oregon and MitoSciences Inc Eugene Oregon USA

Mitochondrial dysfunction from genetic causes, often mutations in mtDNA, causes a number of early onset diseases such as Leighs disease, MERRF, MELAS and LHON. In addition, slowly declining mitochondrial function, often due to environmental insult in combination with genetic predisposition, is associated with many late onset diseases including Alzheimers, Parkinsons, diabetes and osteoarthritis. The major disfunction is most often altered OXPHOS which both reduces ATP production AND increases the levels of both reactive oxygen as well as reactive nitrative species. We have established simple immunocapture methods to array all five OXPHOS complexes from very small amounts of material in active form for analysis of amounts, enzyme turnover, and post translational modification of each. I will describe the use of this technology for rapidly diagnosing early onset genetic diseases. Recent studies of the alterations in complex I in patients with Parkinsons disease will also be presented including the loss of enzyme acitivity, changed subunit composition and increased modification of the complex by ROS.

Glutathione depletion in Parkinson's disease: potential links between subsequent alterations in mitochondrial structure and function

Julie Andersen, Birgit Schilling, Bharath Srinivas, Steve Danielson, Rebeccah Riley, Shankar Chinta, and Brad Gibson

Buck Institute for Research in Aging, Novato, CA, USA

Glutathione depletion is the earliest detectable biochemical event in the Parkinsonian substantia nigra (SN), occurring prior to selective loss of mitochondrial complex I (CI) activity associated with the disease. Down- regulation ofglutathione levels in cultured dopaminergic cell lines results in decreased mitochondrial function linked to a selective decrease in CI activity (Jha et al., 2000). Loss of CI activity following either acute or more prolonged glutathione depletion appears to be due to reversible nitrosylation of protein subunits comprising this complex. Other alterations which occur as a consequence of glutathione depletion in these cultures include several changes in CI subunit phosphorylation. Identification of these targets will allow us to test their mechanistic involvement in alterations in CI activity and mitochondrial function both in our in vitro model and in transgenic mouse model in which glutathione levels can be titrated within SN dopaminergic neurons in vivo. These studies will ultimately allow us to address not only the mechanisms by which early glutathione depletion may be impacting on mitochondrial structure/function in Parkinson's disease but to address the maintainence of cellular glutathione levels as a possible therapeutic for the disorder.

Transcriptomal and functional consequences of mitochondrial neurodegenerative disease

Gino Cortopassi

University of California, Davis, CA, USA

Mutations in genes expressed in the mitochondria can cause neurodegenerative conditions. We transcriptionally profiled RNA from 9 cell types bearing mutations which confer 5 independent mitochondrial diseases with neurological consequences, including LHON,FRDA,CPEO, MERRF, and NARP, in 22 comparisons of mutants vs controls in each cell type. There was a huge excess of significant genes shared and altered among mutant cells, vs. randomized samples. Genes upregulated in mitochondrial disease include multiple components of the Unfolded Protein Response (UPR) pathway, including ATF4, CHOP and BIP. Several genes in both vesicular secretion and the protein synthetic pathway were down-regulated, and these are known consequences of the UPR. A third category of genes inhibited among mitochondrial diseases was oligodendrocyte-specific genes, including CRMP2, TSPAN3, NPC2, DEGS1, and RAB31. A reconstruction experiment (i.e. inhibition with rotenone), confirmed that mitochondrial inhibition activates the UPR. These data demonstrate that both disease-causing mitochondrial mutations, and biochemical mitochondrial inhibition activate the UPR. UPR activation is known to have deleterious consequences in the white-matter diseases CACH and PMD, consistent with our observation that oligodendrocyte-specific genes are inhibited as a consequence of mitochondrial disease. These results suggest that mitochondrial defects may preferentially cause defects in myelination.

Modulation of mitochondrial function by pro-oxidants: Aging and cardiac ischemia/reperfusion

Luke I. Szweda

Oklahoma Medical Research Foundation

Mitochondria exhibit increased rates of oxygen radical production and age-dependent declines in function in response to cardiac ischemia/reperfusion. Oxygen radicals alter protein function reversibly, indicating the potential for regulation, or cause irreversible damage. Our research seeks to define molecular events responsible for changes in mitochondrial free radical production, biochemical mechanisms by which alterations in redox status modulate mitochondrial function, and factors that shift the balance from reversible inhibition to irreversible inactivation of protein function. Evidence is provided that suggests the following scenario: Increases in mitochondrial calcium concentration induced by myocardial ischemia/reperfusion lead to dissociation of cytochrome c from the inner-mitochondrial membrane. Resulting deficits in electron transport are sufficient to increase the rate of free radical production upon re-oxygenation. Subsequent declines in the ratio of GSH/GSSG result in reversible glutathionylation and inhibition of redox sensitive mitochondrial enzymes. This diminishes the rate of free radical production that may be responsible, in part, for the transient nature of increased free radical production observed during reperfusion. Upon recovery of calcium homeostasis and reassociation of cytochrome c, enzymes are deglutathionylated and activity is restored. Age-dependent increases in the magnitude and duration of mitochondrial calcium- overload induce irreversible dissociation and release of cytochrome c. This promotes irreparable oxidative inactivation of protein and mitochondrial function.

POSTERS

Mild carbon monoxide exposure causes cellular deficits in the cerebellum of developing rats

D. Acuna, I. Lopez, D. Webber, A. Espinosa-Jeffrey, J. Edmond

University of California Los Angeles, California, USA

Pregnant rats were exposed chronically to carbon monoxide (CO) at 25 ppm from gestational day 5 to 20. Then the progeny with their mothers were exposed to CO from postnatal day 5 to 22 days of age. Age matched animals not exposed to CO served as controls. Histological observations at post-natal day P12 and P20 showed Purkinje cells (PC) from CO exposed animals were normal when compared to controls. Neurons and fibers showed decreases in neurofilament proteins in CO exposed pups at P20. Synaptic terminals showed a decrease in synapsin-1 in pups at P12 and P20. Hemeoxygenase-1 and superoxide dismutase-1 were elevated in the molecular layer of CO-exposed pups. By contrast, Calbindin immuno-reactivity in the Purkinje cells was decreased in CO-exposed pups at P12 and P20. In conclusion, these results support our finding that mild chronic CO exposure creates a sustained oxidative stress that impairs the neuronal environment in the developing cerebellum. Oxidative injury is a known component of many human disease conditions, particularly many neurodegenerative diseases. The cerebellum regulates movement and posture by adjusting the output of the major descending motor systems of the brain. We suggest that CO at very low chronic exposures during development compromises neuronal integrity, which may lead to functional difficulties later in life.

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Evaluation for antioxidant and estrogenic activities of medicinal plant extracts

Ji-Yun Ahn, Sun-Young Choi, Tae-Youl Ha, Sung-Ran Kim

Food Function Research Division, Korea Food Research Institute San 46-1, Baekhyun-dong, Bundang-gu, Seongnam-si, Gyeonggi-do, 463-746, South Korea

Antioxidant and estrogenic activities of ethanol extracts of 46 edible and medicinal plants were evaluated by DPPH radical scavenging assay, TBARS inhibition rate, and yeast transactivation assay respectively. Direct correlation between the DPPH radical scavenging activity and polyphenol content (r^2=0.61) was established through simple regression analysis. But, there was no correlation between TBARS inhibition rate, or transactivation activity and polyphenol content. Among the medicinal plants screened, Glycyrrhiza uralensis F., Rheum undulatum L., and Psoralea corylifolia L. showed strong activities for antioxidant and estrogenic activity. From the results of this study, we selected P. corylifolia L. as a new potential phytoestrogen candidate and identified the estrogenic active compounds. Estrogenic activities of solvent fractions from P. corylifola L. were evaluated by yeast transactivation assay (β-galactosidase assay). Hexane and chloroform fractions among these fractions showed the highest estrogenic activity determined by yeast transactivation assay. Using silica gel chromatography and HPLC for isolation and LC-MS and 1H-NMR for identification, the active component in Psoralea corylifolia L. extract was identified as a bakuchiol.

Molecular effects of fermented papaya preparation on oxidative damage and MAP Kinase activation

O.I. Aruoma, R. Colognato, L. Migliore, Keiko Koike, S. Coecke, E. Lamy, V. Mersch-Sundermann, I. Laurenza, L. Benzi, and M.-C. Lee

London South Bank University, UK, Università di Pisa, Italy, Osato Research Institutes, Gifu, Japan, ECVAM, Ispra, Italy, Justus-Liebig University of Giessen, Germany, Metabolismo Traumatologia e Medicina del Lavoro, Pisa, Italy, Kanagawa Dental College, Kanagawa, Japan

Strategies for the intervention and prevention of cancers, diabetes, cardiovascular disease, HIV/AIDS and diseases of overt inflammation including neurodegenerative diseases, require an understanding of the basic molecular mechanism(s) by prophylactic agents may potentially prevent or reverse the promotion or progression of the diseases (Aruoma et al 2005, Mutation Research 579(1-2) 1-224). The p38 MAP kinase pathway may be an important therapeutic pathway in cardiovascular disease. If either upstream oxidative stress or downstream MAP kinase-mediated signaling cascade is involved targeted pharmacological antioxidant and/or anti-inflammatory interventions may prevent or ameliorate the diverse pathologies. Fermentated papaya preparation (FPP) is a nutitional supplement derived from papaya. FPP had protective role in the oxidative stress induced apoptosis, likely by activating antioxidant intracellular pathways and this may be mediated via the inhibition of the activation of p38 MAPK and Akt phosphorylation. Supplementation of rats with FPP significantly inhibited the increased decay rate constant of the MC-PROXYL ESR in the SHR brain. Thus FPP can modulate oxidative in support of the view that prophylactic potentials in neurodegenerative diseases can be facilitated by FPP.

Early signals of oxidative stress in mice diabetic hippocampus

F. Bosch-Morell[1,2], M. Miranda[1], E. Arnal[1], A. Messeguer[1,3], J. Barcia[1], and F.J. Romero[1,2]

[1] Departamento de Fisiología. Universidad CEU-Cardenal Herrera, Valencia, Spain [2] Fundación Oftalmológica del Mediterráneo, Valencia, Spain
[3] Department of Biological Organic Chemistry, Institut d'Investigacions Quimiques i Ambientals de Barcelona (CSIC), Barcelona, Spain

Introduction: Diabetes mellitus increases the risk of central nervous system disorders such as stroke, seizures, dementia, and cognitive impairment. The mechanisms responsible for the increased risk of these disorders are incompletely understood. One of the mechanisms by which hyperglycemia causes neural degeneration is via the increased oxidative stress that accompanies diabetes. The aim of this work was to confirm oxidative stress in the hippocampus of diabetes mice and to observe the effect of an antioxidant such as CR-6 over it. *Methods*: The diabetes model in mice 21 days after alloxan injection was used to achieve hyperglycemia. Malondialdehyde (MDA), a lipid peroxidation product, concentration was measured by liquid cromatography according to a modification of the method of Richard and GPx activity was measured according to the method of Lawrence et al. Mice were treated daily with 100 mg/kg of CR-6CR-6 (3,4-dihydro-6-hydroxy-7-methoxy-2,2-dimethyl-1(2H)-benzopyran), an α-tocopherol analogue that has shown a potent inhibitory activity against lipid peroxidation in rat liver microsomes and can act as an efficient scavenger of nitric oxide and peroxynitrite. *Results*: MDA concentration in hippocampus was elevated in diabetic animals, confirming the existence of an oxidative burden. GPx activity was assayed in hippocampus and decreased in the diabetic condition. CR-6 administration restored MDA levels and GPx activity in hippocampus.

Amyloid-β peptide binds with heme to form a peroxidase: Relationship to the cytopathologies of Alzheimer's disease

Kathleen Boyle and Hani Atamna

Nutrition and Metabolism Center, Children's Hospital Oakland Research Institute (CHORI), Oakland, CA 94609, USA

Recent research shows that intracellular and oligomeric amyloid-β (Aβ) peptides are the neurotoxic agents in Alzheimer's disease (AD). However, the molecular link between AD and Aβ is not yet fully understood. We have previously proposed a mechanism by which excessive Aβ binds to regulatory heme, triggering functional heme deficiency (HD). The phenotypes caused by HD overlap with the established cytopathologies of AD, e.g. the decline of mitochondrial complex IV, iron accumulation, and oxidative stress. In the current study, we are adding more evidence that Aβ binding to regulatory heme causes HD. We found that heme readily complexes with Aβ, preventing Aβ aggregation. This complex is suggested to deplete regulatory heme, explaining our observed increase in heme synthesis and iron uptake in human neuroblastoma cells treated with Aβ. We also demonstrate a peroxidase activity of the Aβ-heme complex, which catalyzes the oxidation of serotonin and DOPA by H_2O_2. Curcumin, which lowers oxidative stress in the brain of a mouse model for AD, inhibits the Aβ-heme dependent peroxidase. The binding of Aβ to heme supports a unifying mechanism by which excessive Aβ induces HD, causes oxidative damage to macromolecules, and depletes specific neurotransmitters. The relevance of the binding of regulatory heme with excessive Aβ for mitochondrial dysfunction and neurotoxicity and other cytopathologies of AD is discussed.

GSNO induces protein S-nitrosation in isolated intact rat brain mitochondria – Modulation of mitochondrion-driven apoptosis

Allen H.K. Chang, Derick Han, Jerome Garcia,
and Enrique Cadenas

Department of Molecular Pharmacology & Toxicology, School of Pharmacy, University of Southern California, Los Angeles, California 90033, USA

Cysteine S-nitrosation is a post-translational modification that reversibly regulates redox-sensitive proteins. It has been shown to be involved in mitochondrial functions, *e.g.* apoptosis. However, the regulation and the target proteins of such modification in rat brain mitochondria remain elusive. Here we employ the biotin-switch method and show that GSNO, a physiological carrier of NOx (e.g. NO^+), induces protein S-nitrosation in intact rat brain mitochondria and is reversed by exogenous addition of GSH. HPLC analysis suggests that GSH protects mitochondrial proteins by reducing S-nitrosothiols with the formation of GSSG. Interestingly, under our conditions of mitochondria isolation, endogenous GSH was oxidized and deposited as protein-S-SG that can be recovered by the addition of respiratory substrates or DTT. Addition of DTT or respiratory substrates protected proteins from GSNO-induced S-nitrosation that was diminished by the treatment of CDNB (*1*-chloro-*2,4*-dinitrobenzene), an agent that depleted mitochondrial free GSH. LC/MS-MS identified ANT, VDAC, and ATP synthase F_1 complex as targets of GSNO. Intriguingly, ANT and VDAC1 were also found to be S-nitrosated at the endogenous level. Furthermore, GSNO inhibited ATP synthase activity. In summary, extra-mitochondrial GSNO causes significant protein S-nitrosation in rat brain mitochondria with implications in modulating critical functions such as energy production and permeability transition. The level of GSH dictates the susceptibility of these proteins to this modulation. We hypothesize that GSNO may act as a signaling molecule in relaying the message of cytosolic oxidative/nitrosative stress to the mitochondria.

Hypolipidemic effect of dietary quercetin and its glycosides in hamster fed a high fat diet

Il-Jin Cho, Ji Yun Ahn, Sung Ran Kim, Myung-Sook Choi*, Tae-Youl Ha

*Food Function Research Division, Korea Food Research Institute, Seong Nam, Gyeonggi-Do, Korea, * Department of Food Science & Nutrition, Kyungpook National University, Daegu, Korea*

This study was conducted to evaluate the hypolipidemic effect of dietary quercetin and its glycosides by various biomarkers. Seventy male Syrian Golden hamsters were divided by randomized block design into seven groups; normal group(N), high fat diet group(C), 0.025 fenofibrate(P), 0.05% quercetin(HQ), 0.025% quercetin(LQ), 0.025% quercetin 3 glucoside(Q3G) and 0.025% quercetin rutinoside(RT). They allowed free access to feed for 8 weeks. Concentrations of serum total cholesterol and apo B were significantly lower in all experimental group compared to the high fat control group. Atherogenic index (AI) was also decreased by quercetin and its glycosides. The most reduction was shown in LQ group. The decrease of CETP was shown in only LQ diet group. The levels of apo A-I and HDL-cholesterol in LQ and Q3G group were higher than those in C group. The contents of liver triglyceride were significantly reduced in HQ and LQ groups compared to the control, and these results were reconfirmed by ORO staining of liver tissue. LQ diet increased serum total antioxidant status and decreased TBARS contents significantly. The activities of liver ACAT and HMG-Co A reductase were not changed by the supplementation of quercetin. HMG-Co A reductase mRNA expression was also not changed. These results suggest that dietary quercetin may be a more effective hypolipdemic agent than it glycosides.

Reduced isoalpha acids from *Humulus lupulus* attenuate TNFα signaling in adipocytes

Gary Darland , Matthew L. Tripp, Linda M. Pacioretty, Jeffery S. Bland, John G. Babish

Metaproteomics, Gig Harbor, WA, USA

Increased plasma concentrations of inflammatory cytokines, particularly TNFα, have been associated with obesity, diabetes and metabolic syndrome. Decreasing the pro-inflammatory effects of TNFα stimulation of adipocytes can improve insulin action. Our laboratory previously characterized the anti-inflammatory activity of a series of compounds isolated from hops. Screening these and 203 commercial botanical products using insulin resistant 3T3-L1 adipocytes identified potent lipogenic activity associated with reduced isoalpha acids (RIAA). Further testing with 3T3-L1 adipocytes indicated RIAA inhibited TNFα-induced lipolysis and NF-κB-mediated IL-6 secretion. Additionally, RIAA enhanced adiponectin secretion in TNFα-stimulated 3T3-L1 cells. When administered to db/db diabetic mice, RIAA decreased non-fasting serum insulin. To elucidate the effects of RIAA on insulin signaling in adipocytes, mature 3T3-L1 cells were treated with 5 μg RIAA/mL for 5 hrs, stimulated with 20 ng TNFα overnight followed by 100 nM insulin for 15 min. Aspirin at 5 mM was used as a positive control. RIAA had no effect on insulin receptor tyrosine phosphorylation, decreased p-serine307IRS1/2, increased p-PI3K, increased p-Akt, increased p-MEK1/2, decreased p-JNK and decreased nuclear NF-κB. Taken together these results indicate RIAA functions to enhance insulin induced PI3K/Akt signaling and attenuate TNFα signaling through the NF-kB pathway in the adipocyte.

Protection of vascular endothelial cells from oxidative damage by oligomeric proanthocyanidins

B. de Haan, S. Bapat* and J.A. Post

Cellular Architecture & Dynamics, Utrecht University, Utrecht and
**International Nutrition Company, The Netherlands*

Reactive oxygen species (ROS) are involved in the pathology of cardiovascular conditions such as inflammation, atherosclerosis etc. ROS damage to endothelial cells that line vasculature results in endothelial dysfunction, which plays an important role in the onset and progression of vascular disease. This study investigates the ability of oligomeric proanthocyanidins (OPCs) to protect vascular endothelial (VE) cells from oxidative damage. OPCs were extracted from *Vitis vinifera* seeds and the product, provided by International Nutrition Company, comprised catechins and multimers of 2-5 flavan-3-ol units, as verified by HPLC. VE cells were grown for various times in the presence of OPCs, after which the medium was washed-off. After exposure to hydrogen peroxide (H_2O_2), cell viability and integrity were measured. A time- and dose- dependent attenuation of cell death was observed. As an example: pre-incubation with OPCs (60 μg /ml, 24 hrs) resulted in a reduction of H_2O_2-induced cell lysis from 70 to 10%. Pre-incubation of the H_2O_2 containing medium with OPCs and subsequent incubation of the cells with the mixture also resulted in an OPCs dose dependent attenuation of cell death. The results show that i) OPCs attenuate the effect of oxidative stress when present at the same time and ii) pre-incubation of cells with OPCs renders the cells less susceptible towards oxidative stress. The latter indicates that OPCs, directly or indirectly, enhance the antioxidant status of vascular endothelial cells.

Obesity, dietary antioxidants and ischemic stroke: A case control study

A. De Martino[1,2], G. Cairella[1], F. Garbagnati[1], M. Multari[3], U. Scognamiglio[1] V. Venturiero[3], S. Paolucci[3]

[1]CESAR, IRCCS Fondazione S. Lucia, Rome–Italy- 2University "Tor Vergata", Rome–Italy 3IRCCS Fondazione S. Lucia, Rome–Italy

Evidence that nutritional factors influence the incidence of stroke is growing. Daily intake of fruits and vegetables, well-known rich in antioxidants, may decrease the risk of stroke. A positive connection between obesity and stroke has also been suggested. We carried out a case control study to examine the association between obesity, antioxidants intake and ischemic stroke in Italian subjects. The subjects at first ischemic stroke (n=40; 65.2±13.0 years; 20 males and 20 females) were enrolled and matched by age, sex and smoking status and compared with stroke-free controls subjects (n=40; 65.2±13,29 years). BMI was calculated and a food frequency questionnaire (193 items) was used to evaluate daily food intakes of fruits and vegetables, total antioxidants, vitamin C and β−carotene during the previous years. The rate of overweight/obesity is higher among post-stroke subjects (BMI 25.48±3.93 vs. 24.24±3.87; p=0.02). Using a weighted multivariate logistic regression, dietary intake of fruit and vegetable (OR 0.22; CI 0.05-0.93), total antioxidants (OR 0.14 CI 0.02-0.89), vitamin C (OR 0.56; IC 0.17-1.88) and β carotene (OR 0.40; IC 0.11-1.39) were inversely associated with ischemic stroke; obesity was not significantly associated with ischemic stroke (OR 2.16; CI 0.96-5.91). *Conclusion* - These observations suggest that high dietary intake of antioxidants, mainly from fruit and vegetable may help to prevent ischemic stroke. The association between obesity and stroke remains controversial.

Diagnosis of lipid or carbohydrate susceptible phenotype/genotype in prevention of metabolic syndrome

Dembinska-Kiec A, Wybranska I., Partyka L., Kiec-Wilk B., Malczewska-Malec M., Golabek I.

Department of Clinical Biochemistry, Jagiellonian University, Medical College, Krakow, Poland

Background: Nutrigenomics, the discipline studying the interactions between nutritional factors, genetic background and health goals to achieve more efficient individual dietary intervention strategies aimed at prevention disease, improve quality of life and healthy aging.

*Methods***:** Within the 308 patients with familiar obesity the estimation of 18 more common of "obesity risk-genes" polymorphisms and phenotyping (including the insulin and lipids after glucose tolerance test (OGTT) and lipid tolerance test (OLTT)) was performed.

*Results***:** We demonstrated, that the OLTT/OGTT insulin output ratio (OIOR) segregate the gene variant carriers into the groups of the more glucose- or the more lipid - dependent phenotypes. The mean value of such index is 2,34, [values varied from 0,56 to 8,02] and is not dependent of sex. The high OIOR value pointed to the worse tolerability of lipids than of glucose content in the meal. Screening the genotypes according to those index, the highest value of OIOR in FoxC2 TT genotype carriers, and inversely the lowest value in the FABP-1 GG genotypes carriers.

Conclusions: The value of the OLTT/OGTT insulin output ratio (OIOR) seems to have the predictive value for discrimination between the phenotype susceptible for adipogenic activity of fat or carbohydrate containing diet. The identification of common genetic variants connected with increased risk of high or low OIOR may optimize the use of medical resources through early identification of sub-populations at risk and targets groups for early prevention.

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Procyanidins could act as antioxidant protectors of the gut epithelium

A.G. Erlejman[1], C.G. Fraga[2,3] and P.I. Oteiza[1,3,4]

[1]IQUIFIB-Dept. Biological Chemistry (UBA-CONICET), [2]Physical Chemistry-PRALIB, School of Pharmacy and Biochemistry, UBA, Argentina; [3]Department of Nutrition and [4]Environmental Toxicology, University of California, Davis, CA, USA

Large procyanidins are potent natural antioxidants acquired with the diet. However due to their high molecular weight they are not absorbed, and are poorly degraded by the intestinal microflora. The aim of this work was to investigate the capacity of hexameric procyanidins to interact with intestinal cells. Caco-2 cells forming an epithelial monolayer were incubated in the presence of a fraction of hexamers isolated from cocoa. Hexamers interacted with the cell plasma membrane as evidenced by the increase in the transepithelial electrical resistance of the cell monolayers. At physiologically relevant concentrations, hexamers protected cells from: a) the oxidation and loss of barrier integrity mediated by the lipophilic free radical generator (2,2'-azobis (2,4-dimethylvaleronitrile, AMVN); b) the cytotoxicity and loss of barrier integrity promoted by bile salts; and c) the TNFα- and IL-1-induced activation of NF-κB. Under the current experimental conditions, hexamers did not affect the integrity and functionality of Caco-2 monolayers. Results show that hexamers can interact with Caco-2 cell membranes and act as antioxidant, protecting cells from different extra cellular cytotoxic and pro-inflammatory stimuli. At the oral cavity and gastrointestinal tract, large procyanidins could exert beneficial health effects in pathologies such us inflammatory diseases, alterations in the intestinal barrier permeability and cancer.

Pyruvate normalized excessive formation of reactive oxygen species by increase of NO production during graduate treadmill exercise

Bruno Fink*, Olaf Sommer**

**Noxygen Science Transfer & Diagnostics GmbH, Germany; **Department of General and Visceral Surgery, University of Freiburg, Germany*

Treadmill exercise initiates formation of reactive oxygen species (ROS). This leads to oxidative stress and cellular damage, if not appropriately counteracted. The objective of this study was to compare in vivo formation of ROS with hemodynamic parameters at rest, as well as during graduate treadmill exercise after change of intracellular NAD/NADH ratio by administration of pyruvate. Methods: Dogs were exercised at 5, 10 and 15 km/h (5 % grade), which is equal to strain of 0.4, 1.2, 1.7 joules. In vivo generated ROS were scavenged using of spin probe CPH and quantified by ESR. Production of nitric oxide with and without pyruvate treatment (5 mM) was detected in cultured endothelial cells (ECs) using ESR and colloid Fe(DETC)2 spin trap. Results: Arterial systolic pressure (sBP), heart rate (HR), and ROS formation increased significantly with graduation of exercise. Concomitant, continuous I.V. administration of pyruvate (8 mg/kg/min) during graduate treadmill exercise significantly ($p < 0.05$) diminished increase in sBP, HR, and ROS formation up to 14, 16, 21%, respectively. These results correlate with a decrease in pulse width, and with decrease in formation of lactate. Therefore, we observed elevation of plasma antioxidative capacity reflected by an increase from 178±28 to 205±27 mM in reduced SH-groups concentration under concomitant pyruvate administration. Addition of pyruvate to cultured ECs resulted in up to 32 % increase of NO production. Conclusion: Enhanced formation of ROS during graduate treadmill exercise can be lowered up to 30% by the change of intracellular NAD/NADH ratio, endothelial nitric oxide formation, and elevation of the antioxidative capacity of plasma by administration of pyruvate, correlating with a decrease in lactate formation.

Transcriptional regulation of peroxiredoxin-6 in mouse liver cells

Bridget Gallagher, Carolyn S. Stankiewicz & Shelley A. Phelan

Fairfield University, Fairfield, CT, USA 06430

Peroxiredoxin 6 (Prdx6) is a member of the thiol-specific family of antioxidants, which reduce cellular peroxides using their conserved cysteine residues. We have previously demonstrated that Prdx6 is widely expressed, with very high levels in liver, and is capable of protecting cells from oxidative damage. In the present study we investigated the transcriptional regulation of Prdx6 in the mouse H2.35 hepatocyte cell line in response to various stimuli, and sought to determine the mechanism of basal and induced expression. We found that Prdx6 expression is down-regulated upon serum deprivation, and subsequently induced in a time-dependent manner in response to keratinocyte growth factor (KGF), tumor necrosis factor-α (TNF-α), and more weakly in response to dexamethasone and hydrogen peroxide. Induction peaks between three and eight hours, and returns to basal levels by 24 hours. We next examined potential signaling molecules that may mediate Prdx6 induction. We found that chemical inhibition of PKC prevented Prdx6 induction by KGF, but not TNF-α, while inhibition of NF-κB actually led to a superinduction by both KGF and TNF-α. These data suggest that PKC may mediate KGF-induced expression of Prdx6, while NF-κB may suppress it. We additionally generated and tested reporter constructs containing different regions of the Prdx6 promoter to identify regions responsible for basal and induced transcription. Together, these data will help to elucidate the transcriptional regulation of this important antioxidant, and its role in cell growth and the stress response.

Glutathionylation as a determining factor in redox status and modulator of mitochondrial bioenergetics

Jerome Garcia[1], Derick Han[2], Neil Kaplowitz[2], and Enrique Cadenas[1]

[1] *Molecular Pharmacology & Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90089-9121, USA*
[2] *Research Center for Liver Disease, Keck School of Medicine, University of Southern California, 2011 Zonal Avenue, Los Angeles, CA 90089-9121, USA*

Glutathione, the most abundant non-protein thiol in the cell, plays a major role in regulating biochemical processes that are sensitive to the cellular redox status. Cellular glutathione is found in both the cytoplasm and mitochondria. Interestingly, depletion of the mitochondrial pool of GSH, rather than cytoplasmic, has more profound consequences with respect to cell viability and/or dysfunction. This may be in part due to oxidation of protein sulfhydryl groups, e.g. thiolation and glutathionylation, of mitochondrial proteins. Glutathionylation, a post-translational redox-dependent modification, is an expanding field, however little is known about its effects with regards to mitochondrial function. This present study focuses on GSH and GSSG content in brain and liver mitochondria with respect to different isolation methods, specifically discontinuous percol gradient and differential centrifugation. This study shows that variations in isolation methods and substrate supplementation significantly affected both the glutathionylation of mitochondrial proteins and the redox status of mitochondria, this in turn affected mitochondrial bioenergetics through the modulation of ATP synthase activity.

Antioxidants in sea bass development: The glutathione peroxidase defence

Guerriero, G., Annona G., Ferro R., Villano E. and Ciarcia G.

Department of Biological Sciences, "Federico II" University, Via Mezzocannone 8, 80134 Napoli, Italy

During hatching and development qualitative and quantitative pro-oxidant changes occur, linked especially to respiration rates, tissue reorganization and development. To maintain health and prevent oxidation-induced lesions and mortalities, there must be effective antioxidation systems operating in fish, as in other vertebrates. Compounds against lipid peroxidation, such as carotenoids, vitamins and low molecular weight scavengers, were found in eggs and larvae. In the overall objective of our experimental design related to the important role in understanding the origin and the formation of protective mechanism during the life history of organism, we chose for our investigation the sea bass, Dicentrarchus labrax, a widely utilised species in aquaculture. In this work we analysed the sea bass selenoprotein glutathione peroxidase (GPx) activity, after microscopic analysis, in seminal fluid and eggs, in developing embryos and larvae. GPx was found in seminal fluid, in eggs before and after fertilization, in embryos during development, at hatching and during larval development. The content was very low in segmentation and organogenesis whereas increased at the end of embryogenesis and in larval development. This study provides evidence on how selenoproteins as GPx exert an antioxidant defence in sea bass development. G.Guerriero & G. Ciarcia. Biomarkers of stress and reproduction in fish.

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LDL protein nitration: A model for LDL protein unfolding and LDL$^-$ formation

Ryan T. Hamilton, Derick Han, Liana Asatryan, Juliana Hwang, and Enrique Cadenas

Department of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA

Modification/oxidation of low density lipoprotein (LDL) contributes to the development of atherosclerosis. Increased production of peroxynitrite from the reaction of nitric oxide (from iNOS) and superoxide (from NADPH oxidase) may play an important role in protein nitration and lipid modification of LDL in atherosclerosis. It has been shown that atherosclerotic lesions have increased levels of labile iron which is a strong catalyst of protein nitration by peroxynitrite. However, the effects of protein nitration on LDL structure have not been extensively studied. Fe-catalyzed LDL modification was greater than peroxynitrite alone at protein nitration, lipid peroxidation, and LDL$^-$ formation and percent LDL$^-$ was related to nitrotyrosine (NT) levels. Circular dichroism (CD) spectra of in vivo LDL$^-$ and native LDL were similar for Fe catalyzed and non catalyzed peroxynitrite treated LDL with a decreased α-helical structure and increased β-strand/random coil structure. Total LDL CD spectrum for in vivo LDL was different from Fe-catalyzed peroxynitrite and peroxynitrite-treated total LDL and was dependent on the percent LDL$^-$. Mass spectrometry data show that apoB-100 has moderate α-helice nitration and minimal β-sheet nitration and the LDL$^-$ subfraction had the highest percent nitration (NT/covered tyrosine residues) as compared to native LDL. Our data suggest that nitration of apoB-100 induces its unfolding of α-helices, that nitration of apoB-100 is specific to certain regions of the primary sequence and that this modified particle mimics the structure of in vivo circulating LDL$^-$.

Hydrogen peroxide and redox modulation sensitize to TNF-induced apoptosis of primary mouse hepatocytes

Derick Han, Naoko Hanawa, Behnam Saberi, and Neil Kaplowitz

USC Keck School of Medicine 2011 Zonal Ave, Los Angeles, CA 90033 - USA

Tumor necrosis factor (TNF) plays an important role in mediating hepatocyte injury. TNF treatment alone does not cause death of primary hepatocytes, suggesting other factors are necessary to mediate TNF-induced injury. In this work the question of whether reactive species (ROS) can sensitize primary cultured hepatocytes to TNF-induced apoptosis was investigated. Sublethal levels of H_2O_2, either as bolus doses or steady levels generated by glucose oxidase, were found to sensitize cultured hepatocytes to TNF-induced apoptosis. High levels of H_2O_2 also triggered necrosis in hepatocytes regardless of whether TNF was present or not. Similarly, antimycin, a complex III inhibitor that increases ROS generation from mitochondria, sensitized hepatocytes to TNF-induced apoptosis at low doses but caused necrosis at high doses. Redox changes appear to be important in sensitizing primary hepatocytes, since diamide, a thiol oxidizing agent, and BCNU, an inhibitor of GSSG reductase, also increased TNF-induced apoptosis in hepatocytes at sublethal doses. Agents that sensitized hepatocytes to TNF-induced apoptosis all caused a dramatic fall in the GSH/GSSG ratio. These redox alterations were found to inhibit TNF-induced IκB-α phosphorylation and NF-κB translocation to the nucleus, thus presumably inhibiting expression of genes necessary to inhibit the cytotoxic effects of TNF. These results suggest that oxidation of the intracellular environment by ROS or redox modulating agents interferes with NF-κB signaling pathways to sensitize hepatocytes to TNF-induced apoptosis. The implication for liver disease is that concomitant TNF exposure and ROS, either extrinsically generated (e.g. inflammatory cells) or intrinsically generated in hepatocytes (e.g. mitochondria) may act in concert to promote apoptosis and liver injury.

Thioredoxin-1 suppresses the systemic inflammatory responses against cigarette smoking

Tomijiro Hara[1], Atsuyasu Sato[2], Noriko Kato[1], Masahiro Takenaka[1], Hajime Nakamura[3], and Junji Yodoi[1,3]

[1]Department of Biological Responses, Institute for Virus Research, Kyoto University, Kyoto, Japan.; [2]Department of Respiratory Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan.; [3]Department of Experimental Therapeutics, Translational Research Center, Kyoto University Hospital, 54 Shogoin, Kyoto, Japan

Thioredoxin-1 (TRX) is a reduxtion/oxidation (redox) regulating protein with redox active dithiol/disulfide in the active site. TRX shows antioxidative effects and redox-regulating functions. Macrophage migration inhibitory factor (MIF) is a pluripotent cytokine involved in inflammatory and immune responses. MIF also contains redox motif and recently considered as a member of TRX family. Cigarette smoking recruits systemic inflammatory responses and is a major etiological factor of various systemic diseases as well as lung diseases. In the present study, we demonstrate that TRX attenuates the systemic inflammatory responses against cigarette smoking. Real-time PCR showed that the mRNA expressions of tumor necrosis factor alpha (TNF-alpha) and MIF were significantly increased by acute cigarette smoke exposure in the spleen of wild type C57BL/6 mice, whereas they were suppressed in the spleen of TRX over-expressing transgenic mice (TRX-tg), (P=0.025, P=0.05 respectively). These findings suggest that TRX may modulate MIF expression by redox-dependent regulation and suppress the systemic inflammatory responses against cigarette smoking.

The predictive effect of hsCRP, ischemia modified albumin (IMA) levels and the modality of vitamin E and C supplementation on plasma oxidative stress in patients with metabolic syndrome and peripheral atherosclerosis

J.Hartwich, J.Goralska, D.Siedlecka, A.Gruca, M.Trzos, M.Dolecki*, W.Drozdz*, A. Dembinska-Kiec

*Department of Clinical Biochemistry & *2nd Department of Surgery, Jagiellonian University, Medical College, Krakow, Poland*

Plasma hsCRP and ischemia modified albumin (IMA) are cardiovascular risk markers. Vitamin E and C have several antiatherogenic effects. The aim of study was to assess the relation between the way of vitamin intake with plasma oxidant stress and effects of short-term dietary vitamin supplementation on the relation between hsCRP, IMA and plasma oxidative stress/antioxidant capacity.

Vit. E and C (100mg and 200mg bid) were administered during the meal or fasting in healthy controls (n=20), obese/metabolic syndrome patients (n=38) and peripheral vascular disease (PVD) patients after surgical revascularization (n=44) for 14 d in the cross-over study. Plasma hsCRP, IMA, TBARS, LOOH, LDL oxidative susceptibility, FRAP, thiol/albumin ratio, vitamin E, C, redox compensation index were assessed.

Vit. E and C absorption increased and plasma oxidative stress was reduced by food intake. Vascular surgery for PVD patients improved blood rheology but did not reduce oxidative stress. Antioxidant supplementation reduced plasma oxidative stress and improved plasma antioxidant potential more efficiently in subjects in the highest quartile of hs-CRP and IMA levels identified in PVD subgroup compared to control.

Food intake increases the bioavailability of antioxidant vitamins and improves free-radical-scavenging activity. Plasma hsCRP and IMA could serve as potential markers of oxidative stress and select patients requiring supplementation of antioxidants.

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Role of annexin II in estrogen-induced macrophage matrix metalloproteinase-9 activity: The modulating effect of statins

Juliana Hwang, Howard N Hodis, Tzung K Hsiai, Liana Asatryan, and Alex Sevanian

Molecular Pharmacology and Toxicology, University of Southern California, Los Angeles, California, USA

Annexin II (ANXII) is a receptor for tissue plasminogen activator and plasminogen for the conversion to plasmin, which, in turn, induces metalloproteinase-9 (MMP-9). 17β-estradiol (E2) is reported to decrease plasminogen activity inhibitor-1 and increase plasmin and matrix metalloproteinase activity. However, the combined effects of estrogen and statins on macrophage MMP-9 activity and ANXII expression remain unclear. Treatment of J774A.1 macrophages with 1.0 to 100 nM of E2 for 24 hours increased both MMP-9 activity and ANXII expression in a dose-dependent manner ($p<0.05$). Preincubation with EGTA (10 mM) released ANXII from the cell membrane and inhibited the E2-mediated MMP-9 activity as did incubation of macrophages with anti-annexin IgG. In the presence or absence of E2 (5 nM), simvastatin treatment in the range of 0.1 to 5.0 μM significantly reduced macrophage MMP-9 enzymatic activity ($p<0.005$) in a dose-dependent manner. In the presence or absence of E2, simvastatin also decreased ANXII expression ($p<0.05$). These findings indicate that ANXII plays a central role in modulating the enzymatic activity of MMP-9 in response to E2 and that E2-mediated ANXII expression and MMP-9 activity can be prevented by simvastatin. Prevention of E2-mediated activation of MMP-9 by simvastatin suggests that concurrent statin use may account for early event risk of myocardial infarction seen with hormone therapy in recent clinical trials.

Increased brain mitochondrial respiration and function after 24-hour hormone therapy

Ronald W. Irwin, Jon Nilsen, Rana Masri, Sean Iwamoto, Jia Yao, and Roberta Díaz Brinton

Department of Molecular Pharmacology & Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA, USA

Mitochondria are involved in mechanisms induced by hormone therapy (estradiol and progesterone) to prevent toxicity associated with calcium dysregulation and oxidative stress. We have previously shown that 17β-estradiol (E2) and progesterone (P4) afford neuroprotective benefits dependent upon intact mitochondrial function. Further, neuroprotection was found to be associated with increased MAPK activation, CREB activation, bcl-2 expression, and mitochondrial calcium sequestration. In this study, ovariectomized rats were treated with E2, P4 or E2+P4 and forebrain mitochondria were isolated to determine their relative function compared to oil vehicle control. Uterine tissue weight increase due to cell hyperproliferation verified hormone replacement. Isolated mitochondria from hormone-treated rat brain displayed enhanced respiratory function coupled to increased expression and activity of the electron transport chain complex IV (cytochrome c oxidase). Production of H_2O_2 by isolated mitochondria correlated with changes in respiration. We observed an increase in the enzyme activity and expression of cytochrome c oxidase (COX) in primary cultures of rat hippocampal neurons. To determine the site of action for the hormonal regulation of mitochondrial function, we analyzed the expression of nuclear (COX IV) and mitochondrial (COX I, II, and III) encoded genes by real time RT-PCR. Both nuclear and mitochondrial genes displayed robust hormone- and brain region-dependent increased expression. Increased mitochondrial function was not the result of mitochondrial biogenesis, measured by the relative copy number of mtDNA/nuclearDNA. Our future work is aimed at developing hormone therapy regimens that specifically promote beneficial mechanisms of brain mitochondrial function.

Acrolein-induced oxidative stress mediates structural and functional disruption in apolipoprotein E

V. Jairam, S. Tamamizu-Kato, S. K. Hawbecker, and V. Narayanaswami

Children's Hospital Oakland Research Institute, Oakland, CA 94609

It is well established that oxidative damage to proteins such as apoB-100 increases the atherogenicity of low density lipoproteins. However, little is known about the potential oxidative damage to apolipoprotein E (apoE), an exchangeable apolipoprotein that is a resident of very low density and high density lipoproteins. ApoE plays an integral role in lipid metabolism by mediating lipoprotein particle uptake by hepatocytes, thereby lowering plasma cholesterol levels and reducing risk for cardiovascular disease. Hepatic uptake of lipoproteins is facilitated by apoE's ability to specifically bind cell surface heparan sulfate proteoglycans and lipoprotein receptors via basic residues in its 22 kDa N-terminal domain (NT). We investigated the effect of acrolein, product of endogeous lipid peroxidation and a component of tobacco smoke, on the conformation and function of recombinant human apoE3-NT. Acrolein caused oxidative modification of apoE3-NT as indicated by Western blot analysis with acrolein-lysine-specific antibodies and mass spectrometry. Further, acrolein-modification impairs the ability of apoE3-NT to interact with heparin and causes a 5-fold decrease in its ability to interact with lipid surfaces. Circular dichroism and fluorescence spectroscopic analyses revealed secondary and tertiary structural alterations in acrolein-modified apoE3-NT. In summary, our data indicate that acrolein disrupts the structural and functional integrity of apoE3, which likely interferes with its role in regulating plasma cholesterol homeostasis. This has implications regarding the role of apoE in the pathogenesis of oxidative stress-mediated cardiovascular and cerebrovascular diseases, and stroke.

Mechanistic investigation on mitochondrial DNA 8993T>G point mutation-enhanced mitochondrial dysfunction upon visible laser irradiation in NARP cybrids

Mei-Jie Jou[1], Tsung-I Peng[2], and Yu-Huia Wang[1]

[1]Chang Gung University, Tao-Yuan, Taiwan Republic of China, [2]Chang Gung Memorial Hospital, Keelong, Taiwan Republic of China

Mitochondrial DNA (mtDNA) 8993T>G point mutation damages specifically subunit 6 of mitochondrial ATP synthase and is clinically associated with neurological muscle weakness, ataxia, and retinitis pigmentosa syndrome (NARP). How 8993T>G links to mitochondrial and cellular dysfunction was investigated in established NARP cybrids harboring 0% ~ 98% 8993T>G mutation by using single and multiphoton imaging microscopy. Upon laser irradiation, 8993T>G significantly augmented apoptosis via enhanced mitochondrial reactive oxygen species (mROS) generation and mitochondrial calcium (mCa^{2+}) overload, mitochondrial membrane potential (Ψm) depolarization and opening of the mitochondrial permeability transition (MPTP). In triguingly, heterogeneous propagation of mROS and Ψm depolarization within single cell as well as to adjacent cells were observed upon visible laser irradiation. Detail mechanisms were explored including the role of the MPTP, Ψm, mCa^{2+} and mROS formation upon laser irradiation. Laser-induced mROS formation was preceded by an increase of mCa2+. Generation and propagation of mROS were found to be dependent on ER Ca^{2+} with extracellular Ca^{2+} as an original source. Dual measurement of cytosolic Ca^{2+} and mCa^{2+} by Fluo-4 and Rhod-2, respectively, shows mCa^{2+} oscillation was preceded by cytosolic Nimodipine-sensitive Ca^{2+} oscillation. Thus, a direct activation of a Nimodipine-sensitive Ca^{2+} channel by a small amount of mROS generation upon visible laser irradiation may act as a crucial mechanism for visible laser irradiation induced cell death in NARP cybrids.

Mechanistic investigation on visible laser irradiation-induced mitochondrial dysfunction and apoptosis in mtDNA 8993T>G point mutation cybrids

Mei-Jie Jou[1], Yui-Huai Wang[1], Chia-Wei Hsiao[1], Masashi Tanaka[2]

[1]Department Pharmacology, Chang-Gung University, Tao-Yuan, Taiwan,
[2]Department of Genomics for Longevity and Health, Tokyo Metropolitan of Gerontology

Mitochondria in human cells is not only to act as ATP producer through oxidative phosphorylation, but also as many other roles including the modulation of intracellular calcium homeostasis, endogenous producers of reactive oxygen species (ROS) and the regulation of apoptotic cell death. Pathogenic mutation of mitochondrial DNA (mtDNA) is often fatal to cells and can cause a large variety of human mitochondrial diseases. In this study, the syndrome of neuropathy, ataxia, and retinitis pigmentosa (NARP) is characterized by proximal-muscle weakness, sensory neuropathy, developmental delay, ataxia, seizures, dementia, and retinal pigmentary degeneration. This maternally inherited mitochondrial disease is associated with heteroplasmic missense mutations at nucleotide position 8993T>G in the ATPase 6 gene, and leads to a severe impairment of the synthesis of mitochondrial ATP, reducing cellular energy and cell death, particularly in tissues highly dependent upon the oxidative phosphorylation metabolism, such as brain and retina. Precise mechanisms by which mtDNA mutation results in mitochondrial dysfunction and cell death, however, remain unclear. Recently, mitochondria have been implicated as central executioners of cell death and the major source for ROS generation upon environmental stresses. Here we investigate what role does mitochondria play in mitochondrial dysfunction induced by mtDNA 8993T>G point mutation and the underlying mechanisms of mitochondrial dysfunction and how it leads to cell death under mitochondrial specific oxidative stress induced by laser irradiation.

Antioxidants and mood elevators – Heal obesity disease and obesity disorder

Ekta K. Kalra

EK MedExperts Services Pvt. Ltd.Flat No. 302. Krishna Kunj, Gurunanakpura, Nagpur - 440017, Maharashtra, India

Obesity is a condition of health disorder that needs to be answered by many ways. The focus of research and innovation need not stand on oxidative stress alone but should also take mental stress in its area of focus. Excess food consumption as a rule has to be associated with a direct or indirect link to the mental stress or disorder. In normal conditions and with normal priorities in life, why at all the patient would refer to food as the major source of pleasure. This disorientation in the focus of pleasure has to be linked with mental stress. We believe that antioxidants act as mental stress inhibitors too. In these hypotheses, we propose that obesity results from mental stress that needs to be answered by mood elevators and all antioxidants contribute to mood elevation.

Radical-scavenging activity of natural antioxidants having 4-propenylphenol structures

Tomonori Kawashima[1,2,3], Ikuo Nakanishi[1,3], Sushma Manda[1], Yoshihiro Uto[4], Kei Ohkubo[3], Hideko Kanazawa[2], Kiyoshi Fukuhara[5], Haruhiro Okuda[5], Hideko Nagasawa[4], Hitoshi Hori[4], Kazunori Anzai[1], Toshihiko Ozawa[1,6], Shunichi Fukuzumi[3], and Nobuo Ikota[1]

[1]Redox Regulation Research Group, National Institute of Radiological Sciences, Inage-ku, Chiba 263-8555, Japan, [2]Department of Physical Pharmaceutical Chemistry, Kyoritsu University of Pharmacy, Minato-ku, Tokyo 105-8512, Japan, [3]Department of Material and Life Science, Graduate School of Engineering, Osaka University, SORST, Japan Science and Technology Agency, Suita, Osaka 565-0871, Japan, [4]Faculty of Engineering, The University of Tokushima, Minamijosanjima-cho, Tokushima 770-8506, Japan, [5]Division of Organic Chemistry, National Institute of Health Sciences, Setagaya-ku, Tokyo 158-8501, Japan, and [6]New Industry Creation Hatchery Center, Tohoku University, Aoba-ku, Sendai, Miyagi 980-8579, Japan

Natural antioxidants having phenolic structures, such as vitamin E, catechin in green tea, and resveratrol in red wine, are known to show efficient scavenging activities against the reactive oxygen and nitrogen radicals. There are numerous reports, which show the strong antioxidative activities of these phenolic antioxidants *in vivo* and *in vitro*. In this study, we report the radical-scavenging activities of 4-propenylphenol derivatives, such as caffeic acid (CA), artepillin C (AC), reduced artepillin C (ACOH), rosmarinic acid (RA), curcumin (CU), and so on. Addition of ACOH to a deaerated acetonitrile (MeCN) solution of galvinoxyl radical ($GO^{\bullet}$) at 25°C resulted in the decrease in the absorption band at 428 nm due to $GO^{\bullet}$. The second-order rate constant (k_2) for the $GO^{\bullet}$-scavenging reaction by ACOH was determined as $5.1 \times 10\ M^{-1}\ s^{-1}$ from the time course change of the absorption band due to $GO^{\bullet}$. The one-electron oxidation potential (E^0_{ox}) of ACOH was also determined as 1.08 V vs SCE by the second harmonic alternating-current voltammetric measurement in deaerated MeCN. No acceleration effect of magnesium ion (Mg^{2+}) on the k_2 value was observed, indicating that the $GO^{\bullet}$-scavenging reaction by ACOH proceeds via a one-step hydrogen atom transfer from phenolic OH group in ACOH, rather than an electron-transfer oxidation of ACOH. The effect of Mg^{2+} on the k_2 values as well as the E^0_{ox} values of 4-propenylphenol derivatives provides valuable information about the structure-activity and structure-mechanism relationship for the radical-scavenging reactions of 4-propenylphenol derivatives, leading to the development of novel synthetic antioxidants having stronger radical-scavenging activities than natural antioxidants.

Antioxidants:
Trapping free radicals or modulating system reliability?

Vitaly K. Koltover

Institute of Problems of Chemical Physics, RAS, Moscow Region, Russia

Phenolic antioxidants, among them – butylated hydroxytolene (BHT), when regularly introduced into the diet, can increase the lifespan of animals (Harman, 1957). Indeed, these chemicals trap active radicals in the model reactions of free radical peroxidation. However, contrary to the general opinion, BHT, vitamin E, ascorbic acid and other so-called "antioxidants" are unable to operate this simple antioxidant way in vivo, considering that their rate constants and real concentrations are too low to compete with the specialized anti-oxidative enzymes for reactive oxygen species. Meanwhile, of prime importance in keeping the high reliability of biosystems is preventive maintenance (prophylaxis) of their functional elements (Koltover, 2004). Having investigated the effects of BHT on the low-temperature EPR signals of the rat's hearts, we revealed that BHT increases the extent of oxygenation of the tissues. Thereby, BHT performs an indirect anti-oxidative protection, since hypoxia was shown to impair heart mitochondria and trigger the O_2^- production (Nohl, Stolze, Koltover, 1993). BHT was found to induce EPR signal of nitric oxide in the animals' blood along with the dramatic changes in the functions of adenohypophysis, adrenal cortex and thyroid gland. In experiments with Macaca mulatta monkeys, we found a strong positive correlation between the levels of hormones (cortisol, DHEAS) and SOD activity in the animals' blood (Goncharova et al., 2006). We suggest that "antioxidants" extend the lifespan as the mild stressors providing the preventive maintenance against the active forms of oxygen via the NO/hormonal mechanisms.

Correlation of ubiquitin-proteosome system function and neuronal nitric oxidize synthase level to dopaminergic neurons viability

Philip Y. Lam and Enrique Cadenas

Molecular Pharmacology & Toxicology, School of Pharmacy, University of Southern California, Los Angeles, California, USA

Nitric oxide synthase (NOS) is a flavin- and heme- containing enzyme that catalyzes the metabolism of L-arginine to L-citrulline and $^{\cdot}NO$ in the presence of O_2 and NADPH. Neuronal NOS (nNOS) is a Ca^{2+}-calmodulin-dependent isoform of NOS that is constitutively expressed in neuronal cells. It has been well documented that nNOS is proteasomally degraded by the Ubiquitin-Proteosome System (UPS). Various studies have demonstrated that proteasome activity declines with age. Furthermore, dysfunction of UPS is implicated in Parkinson's disease in which the formation of Lewy bodies and a progressive degeneration of dopaminergic neurons are observed. However, there is no publication relating UPS function, ageing and nNOS level to the viability of dopaminergic neuron. Here, we propose that dysfunction of UPS leads to accumulation of nNOS protein and thereby increase the production of reactive nitrogen species (RNS), *e.g.* $^{\cdot}NO$, $ONOO^{-}$, and results in neuronal death due to increased nitrosative stress. In this study, differentiated PC12 dopaminergic cells and rat brains at different age were used to study: *1*) the modulation of nNOS protein by UPS, *2*) the effect of UPS inhibition on neurons viability, *3*) the change of nNOS protein during ageing, and *4*) the change of nitroslyation / nitration level in cytosol and mitochondria in relation to the change in nNOS content. Data obtained in this study raise the possibility that the age-related elevation of nNOS may contribute to the increased nitric oxide-related neuronal cell death.

Dietary modulation of cell signaling pathways: Eicosanoids

Bill Lands

Active eicosanoids are formed in tissues by peroxide-requiring fatty acid oxygenases that act on highly unsaturated fatty acids (HUFA), and the active hormone-like products are rapidly inactivated by dehydrogenases. A balance between these two processes in almost every mammalian tissue gives a wide range of transient regulatory signals via selective cellular receptors. Because dietary supplies control the essential acids available to the oxygenases, they also control the access of derived eicosanoids to receptors with their subsequent signaling actions. Hydroperoxide activation of fatty acid oxygenases has transient dynamics balanced by oxygenase formation and peroxidase removal of needed activators. The faster formation of hydroperoxide activators from n-6 than n-3 substrates sets the stage for major consequences of voluntary food choices on human health. Current average food choices in the USA support excessive n-6 eicosanoid signaling that mediates inflammatory, thrombotic and arrhythmic processes involved in cardiovascular death (http://efaeducation.nih.gov/sig/chainofevents.ppt). The pathological processes can be decreased with pharmacological drugs or with increased competing dietary n-3 fats. Quantitative assessment of the kinetics maintaining n-3 and n-6 acids in tissue HUFA, influencing peroxide tone in tissues and supporting selective receptor activities with eicosanoids can support rational plans to prevent the dietary imbalances that cause death and disability. A distance learning site (http://efaeducation.nih.gov/) provides interactive computerized tools for making personalized food choices (http://efaeducation.nih.gov/sig/kim.html) that meet each individual's sense of taste and risk aversion by finding acceptable foods and estimating the impact of selected specific daily food combinations.

Protective mechanism of Epigallocatechin-3-gallate against *Helicobacter pylori*-induced gastric epithelial cytotoxicity via blockage of TLR-4 signaling

Kee-Myung Lee, Ki-Baik Hahm, Mi-Sun Kwak, and Jae-Yong Chung

Department of Gastroenterology, Genome Research Center for Gastroenterology and liver disease, Ajou University Woncheon-dong Yongtong-gu Suwon, Republic of Korea

(-)-Epigallocatechin-3-gallate (EGCG), one of green tea catechins, is known to suppress H. pylori-induced gastritis through its antioxidative and anti-bacterial actions. In this study, we evaluated protective mechanism of EGCG against H. pylori-induced cytotoxicity in gastric epithelial cells. For analyzing EGCG effect on viability of gastric epithelial cells, MTT assay and dye exclusion assay were performed. The degree of DNA damage was evaluated by Comet assay and apoptotic DNA fragmentation assay. To investigate EGCG effect on H. pylori-induced the toll-like receptors 4 (TLR-4) signaling, RT-PCR and western blot analysis corresponding to glycosylated TLR-4 was done. LOX metabolites were measured with RP-HPLC. Results. EGCG pretreatment effectively rescued gastric mucosal cells from the H. pylori– induced apoptotic cell death and DNA damage, and administration of this catechin enhanced gastric epithelial cell proliferation. H. pylori infection stimulated the glycosylation of TLR-4 which initiates intracellular signaling of infected host cell, and then pretreatment of EGCG completely blocked its glycosylation. The blockage of TLR-4 activation by EGCG resulted in inactivation of ERK1/2 and NF-κB as downstream molecules of TLR-4 signaling induced by H. pylori. This disturbance of H. pylori-induced host cell signaling by EGCG attenuated the synthesis of proinflammatory mediators, HETEs. EGCG pretreatment showed significant cytoprotective effects against H. pylori-induced gastric cytotoxicity via interference of TLR-4 signaling induced by H. pylori.

Characterization of Gpx1, a mitochondrial thioredoxin peroxidase, in *Schizosaccharomyces pombe*

*Si-Young Lee, Eun-Soo Kwon and Jung-Hye Roe

Laboratory of Molecular Microbiology, School of Biological Sciences, Seoul National University, Seoul, Korea

The fission yeast, *Schizosaccharomyces pombe*, has only one glutathione peroxidase homologue (gpx1+) that has non-selenocysteine instead of selenocysteine in the conserved catalytic motif of mammalian system. The null mutation of gpx1 showed sensitive phenotype to various peroxides including phospholipid hydroperoxide at stationary phase. The Gpx1 has a peroxidase activity and the peroxidatic activity involves an intra-molecular disulfide bond between active-site cysteines, Cys36 and Cys82. Gpx1 is reduced by thioredoxin system, indicating that thioredoxin is a real electron donor for Gpx1. Chromosomally integrated GFP fusiondemonstrated that Gpx1 was located primarily in mitochondria. We also observed that gpx1 mutant caused a retarded growth and low respiration rate on glycerol, a non-fermentable carbon source, whereas it grew well as the wild type on glucose, suggesting that some mitochondrial function is compromised. Measurement of membrane potential by fluorescence-activated cell sorting (FACS) indicated abnormal membrane potential of Dgpx1 mutant. The peroxide level of mitochondria in Dgpx1 is slightly higher than that of the wile type, suggesting that Gpx1 could scavenge peroxides in mitochondria. Phylogenic analysis showed that Gpx1 is closely related with mitochondrial GPXs of mammalian. From these results we conclude that Gpx1 in S. pombe serves as a mitochondria-specific anti-oxidative enzyme to defend oxidative stress and to guarantee optimal mitochondrial function.

High-dose vitamin E administration to rats increases its metabolism and excretion

Scott Leonard[1], Eric Gumpricht[2], Michael Devereaux[2], Ronald Sokol[2], and Maret Traber[1]

[1]Linus Pauling Institute, [2]University of Colorado Health Sciences Center

Mechanisms to regulate vitamin E concentrations in plasma and tissues are largely unknown. We devised a method to quantitate tissue tocopherol metabolites, α- and γ-CEHC. Vitamin E supra-enriched livers were obtained from rats that were injected with vitamin E (10 mg RRR-α-T/100 g body weight) daily for 18 days. On days 0, 3, 6, 9, and 18 serum and livers were collected (n=4-8/group), then tocopherols and CEHCs were quantitated using LC-MS. Detection limits of α- and γ-CEHC were 20 fmol, with a linear detector response from 0.025 to 20 pmol injected. Corresponding to an increase in liver α-T from day 0 to day 3 (43 ± 3 to 965 ± 121 nmol/g; $P < 0.001$), liver α-CEHC increased 80-fold (0.2 ± 0.01 to 15 ± 2 nmol/g; $P < 0.001$). Liver α-CEHC remained elevated through day 9, but surprisingly decreased at day 18 (12 ± 2 to 3 ± 0.5 nmol/g, day 9 to day 18 respectively; $P < 0.001$). Consistent with the decrease in liver α-CEHC, serum and liver α-T, and serum α-CEHC, also decreased significantly from day 9 to day 18. Liver α-CEHC concentrations were correlated with serum α-CEHC, liver and serum α-T ($P < 0.001$ for each comparison). α-CEHC represented 0.5 to 1% of the liver α-T concentration. These data suggest that in times of excess liver a-T, increased metabolism of α-T to the more water soluble α-CEHC metabolite occurs. The significant decrease in metabolite production at day 18, despite continued vitamin E administration, suggests that alternative pathways for vitamin E excretion were activated to prevent toxic levels from accumulating.

nSMase2 is activated by tobacco smoke to induced apoptosis in lung epithelial cells

Michal Levy, Elaine Khan, Roni Lanir, S Sianna Castillo, and Tzipora Goldkorn

UCD School of Medicine, Davis, CA, 95616

Cigarette smoke (CS) is the main cause for chronic obstructive pulmonary disease (COPD) and deregulated ceramide signaling is critical for uncontrolled cell apoptosis, a hallmark of pathologic conditions that cause lung injury in these pulmonary diseases.

We have shown that exposure of human airway epithelial (HAE) cells to CS modulates ceramide generation in a similar manner as exposures to H_2O_2, known to be elevated in the breath or serum of patients with COPD. Exposures of HAE cells to different doses of CS for 30 min elevated ceramide generation to the same extent as exposures to the corresponding doses of H_2O_2. On the other hand, silencing of the sphingomyelinase (SMase), nSMase2, eliminated the response to CS exposure, whereas overexpression doubled the response, demonstrating that nSMase2 is the target not only of H_2O_2 but also of CS modulation of SMase activity. Additionally, experiments with HAE cells over-expressing FLAG-tagged nSMase2 showed that exposure to 250 μM H_2O_2 or CS resulted in the preferential translocation of nSMase2 to the plasma membrane, where it can convert sphingomyelin to ceramide. However, exposure to 10 mM GSH resulted in the translocation of nSMase2 to the perinuclear area.

These studies suggest that at the molecular level, there is direct coupling between CS oxidative stress and the ceramide pathway, via a SMase. Under CS exposure a lung SMase, nSMase2, is activated and displays continuous ceramide generation and pro-apoptotic signaling, thus leading to the pathological apoptosis that causes lung injury.

Heme oxygenase-1-derived carbon monoxide upregulates glutathione synthesis in PC12 cells via activation of PI3K/Akt and Nrf2 signaling

Mei-Hua Li[1], Young-Nam Cha[2] and Young-Joon Surh[1]

[1]National Research Laboratory, College of Pharmacy, Seoul National University, Seoul 151-742, South Korea, and [2]Department of Pharmacology and Toxicology, College of Medicine, Inha University, Incheon 382-751, South Korea

Induction of heme oxygenase-1 (HO-1) expression and enhancement of HO activity have been associated with cytoprotection against a wide array of toxic insults. However, the underlying mechanisms responsible for HO-1-mediated cytoprotection remain largely unresolved. In the present study, we investigated the cytoprotective effects of carbon monoxide (CO), one of the products of the HO-1 reaction, against peroxynitrite-induced PC12 cell death. Upon treatment of PC12 cells with the peroxynitrite generator 3-morphoinosydonimine (SIN-1), the expression of glutamate cysteine ligase catalytic (GCLC) subunit, the rate-limiting enzyme in glutathione (GSH) biosynthesis, increased. The SIN-1-induced GCLC upregulation was preceded by induction of HO-1 and subsequent CO production. Inhibition of HO activity by zinc protoporphyrin IX (ZnPP IX) or siRNA knock down of HO-1 gene expression abrogated the upregulation of GCLC expression induced by SIN-1 and reduced the GSH level. In contrast, the CO-releasing molecule (CO-RM) restored the GSH level previously reduced by ZnPP IX. Furthermore, CO-RM treatment upregulated GCLC expression, which was mediated by NF-E2 related factor 2 (Nrf2). CO-RM-induced activation of Nrf2, in turn, was under the control of the phosphotidylinositol 3-kinase (PI3K)/Akt pathway in PC12 cells. In conclusion, these data suggest that CO produced by the upregulated HO-1 rescues PC12 cells from nitrosative stress through induction of GCLC that is mediated by activation of PI3K/Akt signaling and subsequently Nrf2.

Redox ranking of potential inhibitors of angiogenesis, target of cancer treatment

Lichtenberg, M.[1], Dauzonne D.[2], Zoote V.[3], Gerhäuser, C.[1], Bensasson, R.V.[4]

[1]Division of Toxicology and Cancer Risk Factors, German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]UMR 176 CNRS-Institut Curie, Section de Recherche, 26 rue d'Ulm, 75248 Paris Cedex 05, France; [3]Swiss Institute of Bioinformatics, Molecular Modeling Group, BEP-UNIL, CH-1015, Lausanne, Switzerland; [4]Muséum National d'Histoire Naturelle, Laboratoire de Chimie et Biochimie des Substances Naturelles, Case 54, 63 rue Buffon, 75005 Paris, Cedex 05, France

A study of the ability of five 3-nitroflavones (F) to inhibit the onset and progression of colon aberrant crypt foci (ACF) in rats had shown that their efficacy was linearly correlated with their increasing ability to donate electrons, as characterized by the substituent Hammett constants of these flavones [1]. The double aim of the present study was (i) to analyze some cellular aspects of these five agents as inhibitors of endothelial cell functions related to angiogenesis (vessel-formation) which play a major role in cancer progression and (ii) to verify the prediction that a sixth flavone with higher ability to donate electron would have the highest potential to influence endothelial cell functions. The ability to donate electrons was assessed in the present investigation by the energy of the highest occupied molecular orbital E HOMO of each tested agent F, calculated with a simple quantum-mechanical method and representing the energy required to detach an electron from a molecule.

The compounds F inhibited (i) proliferation as well as (ii) migration of microvascular endothelial cells (HMEC-1). The IC_{50} values were linearly correlated with the E HOMO of these six flavones, with respective correlation coefficient of $r^2 = 0.87$ and 0.90. The smaller the absolute E HOMO of an agent F, i.e. the lower its reduction potential, E (F•+/F), the stronger is its electron donor property and the greater were the observed effects.

The redox ranking of the six nitroflavone activities at the cellular level is in agreement with the redox ranking observed *in vivo* for the inhibition efficacy of the onset and progression of ACF in the rat colon [1]. Our observations suggest to further explore the possibility that controlling a defense against carcinogenesis by xenobiotics F can be predicted *via* one physico-chemical parameter, their reduction potential, E (F•+/F).

[1] Steele, V.E., Boone, C.W., Dauzonne, D., Rao, C.V., and Bensasson, R. V. (2002) Correlation between electron-donating ability of a series of 3-nitroflavones and their efficacy to inhibiting the onset and progression of aberrant crypt foci in the rat colon. *Cancer Research,* **62**, 6506-6509

Cigarette smoke (CS) exposure in α-tocopherol transfer protein (TTP) null mice: Effects of γ-tocopherol (GT) supplementation

Yunsook Lim[1], Vihas T.Vasu[1], Kishorchandra Gohil[1], Hnin Hnin Aung[1], Giuseppe Valacchi[1], Maret G. Traber[1,2], and Carroll E. Cross[1]

[1]Department of Medicine, University of California, Davis, CA, and [2]Linus Pauling Institute, Corvallis, OR

CS induces oxidative and nitrosative stress to the respiratory tract (RT) via both oxidants contained in CS and by CS-induced activation of RT inflammatory- immune pro-oxidant processes. CS exposure has been associated with reduced levels of plasma micronutrient antioxidants, in part due to an increased utilization and turnover of alpha-tocopherol (AT) (AJCN 2005;81:95-103). It has been suggested that gamma-tocopherol (GT) may have an expanded spectrum of antioxidant activation compared to AT (Free Radic Biol Med. 2004;1;36(1):1-15). In order to investigate for novel antioxidant functions of GT as compared to AT, wildtype littermates (TTP+/+) and TTP null (TTP-/-) mice (with < 10% of normal plasma and lung tissue AT levels as compared to TTP +/+ mice) were fed (35 mg/kg diet) or GT (1000mg/kg diet) for 8-10 weeks and then exposed to 60 mg/m3 CS, 6 hr/day for 3 days. Endpoints measured included AT and GT levels, GT metabolites (G-CEHC), inflammatory markers (IL-1beta, MIP-2, MMP-9 and COX-2) and oxidative stress markers (4-HNE, HO-1, CuZnSOD and MnSOD). AT and GT selectively modulated CS related inflammatory and oxidative stress markers. The results suggest that TTP-/- mice are more susceptible to CS induced RT inflammation and that GT did not appear to have any apparent protective functions to *in vivo* CS exposure.

Thioredoxin has an important role in preventing autoimmune diseases

Wenrui Liu, Keisuke Shioji, Shin-ichi Oka, Hajime Nakmura, and Junji Yodoi

Department of Biological Responses, Inst. for Virus Research, Kyoto Univ., Kyoto (W.L., S.O., J.Y.); Department of Cardiovascular Med., Graduate School of Med., Kyoto Univ., Kyoto (K.S.); Department of Experimental Therapeutics, Translational Research Center, Kyoto Univ., Kyoto (H. N.), Japan

Thioredoxin (TRX) is a small (12-kDa) protein, plays a variety of redox-related roles. In our present study, we investigated the role of TRX in murine EAM. EAM was generated in BALB/c mice by immunization with porcine cardiac myosin. Animals were randomly divided into 5 groups. Group I was treated with saline day 0 - 20, and sacrificed on day 21; Group II was treated with recombinant human TRX (rhTRX) in saline day 0 - 12, and sacrificed on day 13; Group III was treated with rhTRX in saline day 0 - 12, and sacrificed on day 21; Group IV was treated with rhTRX in saline day 12 - 20, and sacrificed on day 21; Group V was treated with rhTRX in saline day 0 - 20, and sacrificed on day 21. Histopathology analysis showed that only rhTRX treatment from day 0 to 20 ameliorates the disease, while rhTRX treatment of early- or late-phase does not have such an effect. Immunohistochemistry detection of macrophage infiltration, macrophage inflammatory protein (MIP)-1 and MIP-2, and 8-hydroxydeoxyguanosine (8-OHdG) showed that only the whole phase rhTRX treatment significantly suppresses cardiac macrophage infiltration, MIP-1 and MIP-2 expressions, and oxidative damage. Taken together, our results showed that TRX plays an important role during the whole process of murine EAM generation, and attenuates the autoimmune inflammatory responses by its anti-oxidative effects in murine EAM.

Mild carbon monoxide exposure causes deficits in the cochlea of developing rats

I. Lopez, D. Webber, D. Acuna, S. Samandari-Rad,
A. Espinosa- Jeffrey, J. Edmond

University of California, Los Angeles, California, USA

Pregnant rats were exposed chronically to carbon monoxide (CO) at 25 ppm from gestational day 5 to 20. Then the progeny with their mothers were exposed to CO from postnatal day 5 to 22 days of age. Age matched animals not exposed to CO served as controls. Histological observations at post-natal day 3 (P3), P6, P12 and P20 showed a normal complement of inner and outer hair cells in CO exposed pups when compared to controls. Swelling of mitochondria was observed in spiral ganglia neurons of CO exposed animals. The soma and fibers of the spiral ganglia neurons showed decreased neurofilament proteins in CO exposed rats at P20. Synaptophysin and synapsin-1 were decreased in CO exposed pups as early as P6. Two markers for oxidative stress (hemeoxygenase-1 and superoxide dismutase-1) were elevated in the stria vascularis and spiral ganglia neurons. Our results suggest that damage is localized to the spiral ganglia neurons and their terminals. In conclusion, these results support our finding that mild chronic CO exposure creates a sustained oxidative stress that impairs the neuronal environment of the developing cochlea. Oxidative injury is a known component of many human disease conditions, particularly many neurodegenerative diseases. We suggest that CO at very low chronic exposures during development compromises neuronal integrity, and may cause permanent auditory dysfunction throughout life.

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Fractions from *Vaccinium myrtillus* L. fruits in combinations with carotenoids: Study of free radical scavenger properties *in vitro*

Makarova M., Pozharitskaya O., Shikov A., Rydlovskaya A., Makarov V.

Interregional Center "Adaptogen", St-Petersburg, Russia

Aim of work: Study of free radical scavenger properties of fraction from *Vaccinium myrtillus* L. fruits and its combinations with carotenoids in vitro. Methods: Two methods were used to confirm the protective effect of fractions from bilberry fruits. Neutralization of hydroxyl radicals ($HO^{\bullet}$) was estimated by fluorimetric method [1]. Oxidation of plasma was carried out at 37°C by addition of the thermolabile azo initiators 2, 2'-azobis (2,4-dimethylvaleronitrile), which release water- and lipid-soluble $ROO^{\bullet}$ [2]. Results: Crude fraction from bilberry fruits, containing anthocyans is more effective in relation to $ROO^{\bullet}$ radicals (IC_{50} = 0.16 ± 0.004 mg/ml), but less effective in relation to $HO^{\bullet}$ radicals IC_{50} = 0.36 ± 0.003 mg/ml. Hexan fraction from bilberry fruits, containing mainly lipophylic components showed practically identical activity to $ROO^{\bullet}$ (IC_{50} = 0.25 ± 0.03) and to $HO^{\bullet}$ (IC_{50} = 0.27 ± 0.001). Ethylacetat fraction from bilberry fruits, containing polyphenols was 2 times more effective in relation to HO• (IC_{50} = 0.16 ± 0.01), than to $ROO^{\bullet}$ radicals (IC_{50} = 0.34 ± 0.004). It is established, that lutein and zeaxanthin had a significant activity in relation to $HO^{\bullet}$ (IC_{50} = 0.42 ± 0.005 and 0.16 ± 0.007 mM) and $ROO^{\bullet}$ (0.16 ± 0.005 and 0.08 mM). In addition, the activity of double combinations of bilberry fraction with lutein and zeaxanthin was estimated. It is shown, that in relation to $HO^{\bullet}$ all fractions had synergism with carotinoids. In relation to $ROO^{\bullet}$ hexan fraction from bilberry had synergism with zeaxanthin and additivity with lutein, ethylacetat fraction from bilberry on the contrary had synergism with lutein and additivity with zeaxanthin. The crude fraction showed synergism with both carotenoids.

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2. Thomas S.R. et al. (1996)J.Biol.Chem., 271(51):32714 - 32721.

Zinc deficiency-associated increase in oxidant levels affects the translocation of NF-κB and NFAT to the Nuclei by affecting tubulin polymerization

Gerardo G. Mackenzie, Alejandra G. Erlejma, and Patricia I. Oteiza

Departments of Nutrition and Environmental Toxicology, University of California, Davis, USA. Department of Biological Chemistry, School of Pharmacy and Biochemistry, University of Buenos Aires, Argentina

The role of oxidants and tubulin depolymerization on NF-κB and NFAT nuclear translocation associated with zinc deficiency were investigated in human neuroblastoma IMR-32 cells. IMR-32 cells were incubated in control media or chelated media containing 1.5, 5, or 15 μM zinc, without or with 0.5 mM alpha-lipoic acid (LA) or 1 mM N-acetyl-L-cysteine (NAC) for 24 h. Total oxidant cell concentrations were higher and total glutathione concentrations were lower in the low zinc groups (1.5 and 5 Zn) compared to control and 15 Zn groups. LA or NAC prevented the increase in total oxidants levels, and restored glutathione concentration in the low zinc cells. Zinc deficiency induced NF-κB and NFAT activation as measured by EMSA in total cell extracts. However, NF-κB and NFAT accumulated in the cytosol. In the zinc deficient cells, a low rate of in vitro tubulin polymerization and of polymerized tubulin content was observed compared to the other groups, while the simultaneous incubation of zinc deficient cells with LA or NAC partially restored the nuclear translocation of the active NF-κB. In summary, a decrease in cellular zinc affects select transcription factors (NF-κB and NFAT) partially through an increase in cellular oxidants.

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The effect of indomethacin-induced mitochondrial damage, lipid peroxidation, and apoptosis in gastric epithelial RGM-1 cells

Hirofumi Matsui , Yumiko Nagano, Osamu Shimokawa, and Ichinosuke Hyodo

Division of Gastroenterology, Graduate School of Comprehensive Human Sciences, University of Tsukuba

Nonsteroidal antiinflammatory drugs (NSAIDs) cause complications such as gastrointestinal injury. NSAIDs were reported to cause mitochondrial injury: to dissipate the mitochondrial transmembrane potential (MTP), and to induce mitochondrial permeability transition pore (PTP). This enzyme generates reactive oxygen species (ROS) thereby induces cellular apoptosis. However, the mechanism of this NSAID-induced MTP's role remains unknown. Rebamipide, an antiulcer drug, is reported to scavenge ROS and to restrain indomethacin-induced tissue peroxidations. Since ROS are involved in indomethacin-induced cellular apoptosis, rebamipide may attenuate mitochondrial damage. The aim of this study was to elucidate whether indomethacin induces both the MTP decrease and cellular apoptosis, and the effect of rebamipide on these phenomena. We examined the effect of rebamipide on 1) MTP change, 2) lipid peroxidation, 3) apoptosis, and 4) caspase activation using gastric mucosal epithelial cell-line treated with indomethacin. With a specially designed fluorescence analyzing microscope system, MTP change, cellular lipid peroxidation, and cellular apoptosis were investigated with the following fluorescent dyes, MitoRed, DPPP, and Hoechst 33258, respectively. Indomethacin treatment decreased MTP but increased both cellular lipid peroxidation and cellular apoptosis via caspase 3 and 9 activation. Rebamipide clearly inhibited these phenomena in vitro. We demonstrated that fluorescent dyes such as MitoRed, DPPP, and Hoechst 33258 are useful indicators for detecting oxidative cellular injuries in living cells. Rebamipide exerts a protective effect on mitochondrial membrane stability in gastric epithelial cells.

Superoxide production by the mitochondria electron transport chain: Inhibition by calcium

Satoshi Matsuzaki and Luke I. Szweda

Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma

Mitochondria exhibit increased rates of oxygen radical production in response to physiological stimuli and during the progression of numerous degenerative disorders. Declines in the rate of electron transport and increases in the half-life of reduced components of the electron transport chain play a significant role in these processes. We have previously shown that calcium can induce reversible free radical-mediated inhibition of complex I of the respiratory chain. In the current study, submitochondrial particles and specific inhibitors of electron transport chain complexes were utilized to determine the effect of calcium-induced inhibition of complex I on the rate of superoxide production. The results indicate that complex I is the major source of superoxide released outside submitochondrial particles (matrix side of mitochondria) in the absence or presence of complex III inhibitors. Inhibition of complex I by calcium results in a reduction in succinate- and NADH-supported superoxide production. These findings suggest that calcium influences the site of free radical generation directly or indirectly by altering the conformation of complex I. The reversible nature of complex I inhibition indicates that calcium may mediate free radical production in response to physiological and/or pathophysiological conditions associated with alterations in mitochondrial calcium content.

Evidence that antioxidant treatment reduces myocardial infarct size in type 2 diabetes

Paul McDonagh, Raoul Gonzales and Grace Davis-Gorman

Department of Surgery and The Sarver Heart Center, University of Arizona Health Sciences Center, Tucson, Arizona, USA

The interrelationship of type 2 diabetes (T2D), inflammation and ischemic heart disease (IHD) is not clear. A low-level, chronic inflammatory response may aggravate leukocyte-mediated ischemia-reperfusion (I/R) injury in the T2D heart. The purposes of this study were to: 1.) determine if myocardial injury due to I/R was increased in the Zucker Diabetic Fatty (ZDF) model of T2D, 2.) determine if treatment with vitamin E would attenuate ischemic injury. Zucker Lean Controls (ZLC) and ZDF rats were subjected to a left anterior descending coronary artery occlusion-reperfusion protocol. The recovery of left ventricular function and myocardial infarct size were determined. Neutrophil reactive oxygen species (PMN ROS) was measured in whole blood prior to ischemia. Four groups were studied. Group 1: ZLC-Placebo, Group 2: ZLC-Vit E, Group 3: ZDF-P and Group 4: ZDF-E. We found that the recovery of ventricular function (+dP/dt) was somewhat depressed in the diabetic group (ZLC-P = 87.4% ±11.7 SEM, ZDF-P = 68.1% ± 5.5, P=NS), however, the recovery of the ZDF-E group was improved significantly (ZDF-E = 97.4% ± 10.3). The myocardial infarct size was significantly increased in the ZDF-P group but was reduced with vitamin E treatment (ZLC-P = 43.0% ± 1.9, ZDF-P = 57.0% ± 3.9, ZDF-E = 33.9% ± 5.1, $P<0.05$). In addition, vitamin E treatment significantly reduced the chronic increase in PMN ROS observed in the ZDF-P group ($P<0.05$). These findings indicate that ischemic injury is significantly increased in the ZDF heart and is associated with increased PMN ROS. Treatment with the antioxidant, vitamin E reduced both PMN ROS and the severity of IHD in T2D.

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Calorie restriction enhances T cell mediated immune response in overweight men and women

S. N. Meydani, T. Ahemd, S. K. Das, S. B. Roberts, J. K. Golden, and E. Saltzman

Nutritional Immunology Laboratory, JM-USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA, 02111

It is well-known that dietary energy restriction prolongs life-span and enhances immune responsiveness in a wide range of laboratory animals. However, information on the applicability of these results to humans is limited. In this study we examined the effects of calorie restriction on T cell mediated function in humans. Forty-six overweight (BMI= 27.9+1.5 Kg/m^2) men and women aged 35 to 40 years (35+ 5) were randomly assigned to a 30% or 10% (control) calorie restricted groups. Delayed type hypersensitivity skin test (DTH), lymphocyte proliferation, prostaglandin E2 and cytokine productions were determined at baseline and after six months of Calorie restriction. DTH responses, as well as Con A and PHA stimulated lymphocyte proliferation were significantly increased in both calorie restricted groups compared to baseline ($p<0.05$). However, proliferative response to anti-CD3 was increased significantly only in the 30% calorie restricted group. LPS stimulated PGE2 production was reduced in both groups, but reached statistical significance only in the 30% calorie restricted group (33% decrease, $p<0.05$). These results, for the first time, show that 6 months calorie restriction in humans improves T cell mediated function. This effect of calorie restriction is, at least in part, due to decrease in PGE2 production, which has been shown to suppress T cell function.

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Transcriptional regulation of sodium-dependent vitamin C transporter 1

Alexander J. Michels and Tory M. Hagen

Linus Pauling Institute and Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331

Vitamin C is necessary for cellular function, oxidant protection, and signaling events. Any loss of vitamin C uptake, such as that seen in aging, leads to an accumulation of oxidative damage and the progression of disease. Previous results from our lab show that older animals exhibit a preferential loss in mRNA levels for sodium-dependent vitamin C transporter 1 (SVCT1), which may explain alterations in vitamin C status. However, the functional regulation of SVCT1 gene expression has never been explored. Thus, we have characterized the transcriptional control mechanisms of SLC23A1, the gene encoding for SVCT1, using a firefly luciferase reporter gene assay in HepG2 cells. Truncation deletions of the promoter construct have identified two major regions of interest upstream of the transcriptional start point (TSP): a fragment between -240bp and -160bp that shows a repression of transcriptional activity, and the 160bp fragment nearest the TSP that is required for expression of SVCT1. Furthermore, point mutations in this 160bp region have identified that a region identified as an AP-1 binding site that is critical for both the basal transcription of SVCT1. Binding to this specific SVCT1/AP-1 sequence was confirmed by the use of EMSA. Of all compounds tested for SVCT1 stimulation, α-Lipoic Acid (LA) supplementation increased SVCT1 transcription in a time and dose-dependent manner. This stimulation required the presence of a functional AP-1 element, again supporting its importance in SVCT1 transcription. Overall, these novel findings suggest that AP-1 binding and LA are major functional regulators of SVCT1 transcription and play important roles in modulating vitamin C uptake on a cellular level.

Modulation of triglyceride metabolism and glucose homeostasis in fructose-induced insulin resistant hamsters by citrus polymethoxylated flavones

*Rinee Mukherjee, [++]Rachel W. Li, [++]Andre G. Theriault, *Karen Au, *Ling He, [++]Teresa D. Douglas, *Elzbieta M. Kurowska and *Najla Guthrie

**KGK Synergize Inc., London, Ontario, Canada, N6A 5R8 and [++]Division of Medical Technology, University of Hawaii, Honolulu, Hawaii, USA, 96822*

The objective of this study was to determine whether supplementation with Polymethoxylated flavones (PMFs) could ameliorate the fructose-induced hypertriglyceridemia and other metabolic abnormalities associated with insulin resistance (IR) in hamsters. Hamsters were fed fructose-rich diet for 2 weeks thereafter either continued on the same diet with or without PMF-L(low) vs. PMF-H(high) dose for 4 weeks. PMF-treated groups showed a significant decrease ($p<0.05$) in plasma triglyceride (TG) and cholesterol levels compared to the fructose-fed control group. The control group at the end of the study showed elevated serum insulin and impaired insulin sensitivity. On the other hand, PMF-treated groups showed a reversal in these metabolic defects, including a decrease in insulin level and an improvement in glucose tolerance, suggesting anti-diabetic potential. PMFs reduced TG contents in liver and heart tissues, and regulated adipocytokines by significantly suppressing TNF-alpha, IFN-gamma, IL-1beta and IL-6 expression and increasing adiponectin. PMF-H also significantly increased peroxisome proliferator-activated receptors (PPAR)-alpha and PPAR-gamma protein expression in the liver indicating improvement of hepatic TG and glucose metabolism. Thus, dietary PMF supplementation was proven to be effective in preventing hypertriglyceridemia and hyperinsulinemia in a hamster model of IR. A clinical study is currently underway using a synergistic combination of PMFs.

Oxidative stress in the retina in an experimental model of diabetes

M. Muriach[1], M. Miranda[1], F. Bosch-Morell[1,2], E. Arnal[1], S. Asensio[1], and F.J. Romero[1,2]

[1] Departamento de Fisiología. Universidad CEU-Cardenal Herrera, Valencia, Spain [2] Fundación Oftalmológica del Mediterráneo, Valencia, Spain

Introduction: Diabetic retinopathy is the first cause of adult blindness in developed countries, and though strict glycemic control is desirable to prevent complications, this is not always achievable. Thus, adjuvant therapies are needed to help in preventing or delaying the onset of diabetic complications. The biochemical and functional changes in the retina of diabetic mice, and the ability of Lutein (a natural antioxidant) to reverse these effects have been studied, compared to the effect of insulin therapy. Methods: The diabetes model in mice, 7, 14 and 21 days after alloxan injection was used to achieve hyperglycemia. Lutein or insulin was administered daily. Malondialdehyde (MDA), a lipid peroxidation product, concentration was measured by liquid cromatography according to a modification of the method of Richard et al. Glutathione peroxidase (GPx) activity was measured according to the method of Lawrence et al. Serial electrorretinograms (ERG) were recorded Results: GPx activity, the key enzymatic activity metabolizing cytosolic and mitochondrial hydrogen peroxide, was assayed in eye homogenate without lens and decreased after 7, 14 and 21 days of diabetic condition, whereas ocular MDA concentration was higher than controls. ERG amplitude (mostly b-wave) decreased in diabetic animals (7, 14 and 21 days after alloxan injection) respect to controls. Daily lutein administration (100 mg/kg p.o.) or insulin treatment (500 mU/g body weight s.c.), restored MDA levels ocular tissue, the former having no effect on glycemia. Lutein protected retinal GPx activity and restored ERG amplitude in diabetic animals to control values, whereas insulin partially reversed the effects of diabetes on GPx and ERG.

α-Tocopherol modulates hepatic cytochrome P450S and P-glycoproteins

Debbie Mustacich[1], Scott Leonard[1], Mike Devereaux[2], Ronald Sokol[2], and Maret Traber[1]

[1]Linus Pauling Institute, [2]University of Colorado Health Sciences Center

α-Tocopherol (T) does not accumulate during vitamin E supplementation to toxic levels in plasma or tissues suggesting that T is extensively metabolized and/or excreted. To test the hypothesis that T supplementation increases T metabolism by altering hepatic xenobiotic metabolizing systems, rats were injected daily with subcutaneous T (10 mg/ 100 g body wt.) for 18 d and livers were collected every 3 days. By d3, hepatic T increased 70-fold (819 ± 74 nmol/g vs d0, 12 ± 1, $P<0.01$). Hepatic T plateaued then, despite daily T injections, decreased at d9 to 58% of maximum levels and continued decreasing to 40% at d18. Hepatic T transfer protein, important for T secretion into plasma, was unchanged throughout. The major T metabolite, CEHC, increased and decreased similarly to hepatic T, but at 1% T concentrations. Since T metabolism is initiated by cytochrome P450s (CYPs), CYP modulation was investigated in these livers. CYP4F, T- omega hydrolase, was unchanged. Three CYP proteins, involved in the metabolism of 70% of pharmaceutical drugs, increased significantly with T injections, as compared to controls, and remained elevated from the indicated day to d18: CYP3A (at d3, 160% ± 11%), CYP2B (at d3, 185% ± 17%) and CYP2C (at d9, 136% ± 1%). In addition, p-glycoprotein, a biliary transport protein that is often concomitantly modulated with CYP3A, was also up-regulated by T. These studies have clinical significance for humans taking therapeutic drugs and vitamin E, in that T could hypothetically alter the bioavailability and thus the efficacy of therapeutic drugs.

Synthesis and radical-scavenging activity of planar catechin derivatives having alkyl side chains

Ikuo Nakanishi[1,2], Tomonori Kawashima[1,2,3], Ayako Tada[4,5], Haruko Yakumaru[1], Kei Ohkubo[2], Hideko Kanazawa[2], Shiro Urano[4], Haruhiro Okuda[5], Naoki Miyata[6], Kazunori Anzai[1], Toshihiko Ozawa[1,7], Shunichi Fukuzumi[2], Nobuo Ikota[1], and Kiyoshi Fukuhara[5]

[1]Redox Regulation Research Group, National Institute of Radiological Sciences, Inage-ku, Chiba 263-8555, Japan, [2]Department of Material and Life Science, Graduate School of Engineering, Osaka University, SORST, Japan Science and Technology Agency, Suita, Osaka 565-0871, Japan, [3]Department of Physical Pharmaceutical Chemistry, Kyoritsu University of Pharmacy, Minato-ku, Tokyo 105-8512, Japan, [4]Department of Applied Chemistry, Shibaura Institute of Technology, Minato-ku, Tokyo 108-8548, Japan, [5]Division of Organic Chemistry, National Institute of Health Sciences, Setagaya-ku, Tokyo 158-8501, Japan, [6]Department of Organic and Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Aichi 467-8603, Japan, and [7]New Industry Creation Hatchery Center, Tohoku University, Aoba-ku, Sendai, Miyagi 980-8579, Japan

The development of novel antioxidants having enhanced radical-scavenging activities as compared to natural antioxidants has attracted considerable interest to remove reactive oxygen species (ROS), such as $O_2^{\bullet-}$ and $^{\bullet}OH$, which are known to induce oxidative stress and associated diseases. Recently, we have reported that a planar catechin derivative (C1), synthesized in the reaction of (+)-catechin (C0) with acetone in the presence of $BF_3{\bullet}Et_2O$, shows an enhanced protective effect against the oxidative DNA damage induced by the Fenton reaction without the pro-oxidant effect, which is usually observed in the case of C0. The spectroscopic and kinetic studies have demonstrated that the rate of the scavenging reaction of galvinoxyl radical ($GO^{\bullet}$) by C1 is about 5-fold faster than that by the native C0 in deaerated acetonitrile (MeCN). We have also demonstrated that the $O_2^{\bullet-}$-generating ability of the dianion form of C1 generated in the reaction of C1 with two equivalents of Bu_4NOMe in deaerated MeCN is much lower than that of C1, suggesting that C1 may be a promising novel antioxidant with reduced pro-oxidant activity. In this study, we have synthesized 8 planar catechin derivatives with different lengths of alkyl side chains by using $[CH_3(CH_2)n]_2CO$ (n = 1–8) instead of acetone. It was found that the larger the number of the carbon in the alkyl side chains is, the faster the $GO^{\bullet}$-scavenging rates become. The mechanistic investigation of such acceleration by the alkyl side chains will also be presented.

Thioproline dietary supplementation decreases spontaneous food intake and increases survival and brain function in mice

Ana Navarro[1], Maria Jesus Sanchez-Pino[1], Carmen Gomez[1], Manuel J. Bandez[1], Enrique Cadenas[2] and Alberto Boveris[3]

[1]Department of Biochemistry and Molecular Biology, School of Medicine, University of Cádiz, 11003-Cádiz, Spain, [2]Department of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90089-9121 and [3]Laboratory of Free Radical Biology, School of Pharmacy and Biochemistry, University of Buenos Aires, C1113AAD Buenos Aires, Argentina.

Male mice supplemented with thioproline (*l*-thiazolidine-4-carboxylic acid), a physiological metabolite of 5-hydroxytryptamine, at 2.0 g/kg of food and from 28 weeks of age and for the rest of mice lives, showed a 29 % increased median life span (from 62 to 80 weeks) and a 23% increased maximal life span (from 118 to 145 weeks), associated with improved neurological functions as assayed by the tightrope and the T-maze tests, at 52 and 78 weeks of age. Thioproline-supplemented mice had, compared with control mice, a 20% lower integral spontaneous food intake and a 4-10% lower body weight between 52 and 100 weeks of age. The body weight of control and thioproline-treated mice showed an statistically significant inverse relationship with survival and neurological performances. Mice supplemented with thioproline exhibited a 58-70% decreased age-dependent oxidative damage in brain and liver mitochondria between 52 and 78 weeks of age. The age-associated decrease in brain mitochondrial enzyme activities (NADH-dehydrogenase, cytochrome oxidase and mtNOS) observed between 52 and 78 weeks was partially prevented (51-74%) by thioproline intake. Thioproline *in vitro* neither exhibited direct antioxidant activity nor had any effect on the electron transfer and mtNOS functional activities of brain and liver mitochondria. Thioproline induces an anorexic effect, associated with improved survival and neurological function through a physiological mechanism that likely involves hypothalamic appetite centers.

Protandim™: A fundamentally new approach to antioxidant therapy

Sally K. Nelson, Swapan K. Bose, Jay Chow, Lee Chow, Gary K. Grunwald and Joe M. McCord

Division of Pulmonary Science and Critical Care Medicine and Department of Preventive Medicine and Biometrics, University of Colorado Denver Health Sciences Center, Denver, CO, USA

AA composition of plant extracts (Protandim™), chosen for their abilities to induce superoxide dismutase and catalase was first administered to C57/B6J mice for 23 days. Four groups of mice received the composition at 0, 16, 48, or 160 ug/gbw/day in their food for 23 days. SOD activity was significantly induced in liver by up to 45% and in RBC by up to 25% in a dose-dependent manner. More importantly, lipid peroxidation products as thiobarbituric acid-reacting substances (TBARS) decreased by 60-95% in plasma, liver and brain in a significant dose-dependent manner. A reformulated and improved composition was then used in healthy human subjects ranging in age from 20 to 78 years. Blood was drawn and analyzed from each subject prior to supplementation, and after 30 and 120 d of supplementation (675 mg/d). Erythrocytes were assayed for SOD and catalase, and plasma was assayed for TBARS, uric acid, C-reactive protein, and cholesterol (total, LDL, and HDL). Prior to supplementation, TBARS showed a strong age-dependent increase. After 30 d of supplementation, TBARS declined by an average of 40% ($p < 0.0001$) and the age-dependent increase in TBARS seen prior to supplementation was eliminated. By 120 days, erythrocyte SOD increased by 30% ($p < 0.01$) and catalase by 54% ($p < 0.002$). We conclude that modest induction of the catalytic antioxidants SOD and catalase may be a much more effective approach than supplementation with conventional antioxidants (such as vitamins C and E) that can, at best, stoichiometrically scavenge a very small fraction of total oxidant production.

Synergism of *Helicobacter pylori* infection and stress on augmentation of gastric mucosal damages and prevention with α-tocopherol

Tae Young Oh, Marie Yeo, Sang Uk Han, Yong Kwan Cho, Young Bae Kim, Yong Seok Kim, Sung Won Cho, Ki-Baik Hahm

Genomic Research Center for Gastroenterology, Ajou University School of Medicine, Suwon, South Korea

We identified that oxidative stress played a critical role in the augmented mucosal damages of stress in *H. pylori* infection and that α-tocopherol could ameliorate the aggravation of gastric mucosal damage. The rats were divided into two groups according to *H. pylori* inoculation, and after 24 weeks of infection, the water immersion restraint stress (WIRS) was imposed for 30, 120, or 480 min, respectively. To evaluate the therapeutic effect of α-tocopherol was administrated 40 mg/kg daily prior to imposing WIRS. Remarkably increased hemorrhagic lesions and bleeding index were noted in *H. pylori* infected group with statistical significance ($p<0.05$) compared to non-infected group. Significantly higher oxidative stress documented by iNOS, lipid peroxides and GSH level were detected in *H. pylori* infected group. Proteomic analysis showed the decrease of HSP 27 and other chaperon proteins. Pretreatment of α-tocopherol significantly prevented the gastric mucosal damages caused by *H. pylori* / WIRS. α-tocopherol induced HSP27 expression, which was well correlated with down-regulation of iNOS mRNA. Conclusively, the presence of *H. pylori* caused significant deterioration of stress-induced gastric mucosal lesions through the increased oxidative stress and antioxidant treatment such as α-tocopherol protected the gastric injuries.

Modulation of lung cytoskeleton gene expression in response to α-tocopherol in C57BL6 mice

S.Oommen[1], VT.Vasu[1], RO.Balza Jr.[2], SW.Leonard[3], MG.Traber[3], CE.Cross[1], and K.Gohil[1]

[1]Department of Int Med, UC Davis, [2]Wisconsin Lutheran College, Milwaukee, [3]Linus Pauling Institute, Oregon State University, Corvallis

α-tocopherol (AT) is the most abundant member of vitamin E family in mammalian tissues. It has antioxidant and non-antioxidant actions *in vivo*. Its actions on affecting mRNA homeostasis are the focus of the present study. Changes in the expression of ~15,000 mRNAs from male and female mouse lungs were screened in response to dietary AT. Mice were fed either a minimal (<10 U/kg diet), a basic (35 IU/kg diet) or a high (1000 IU/kg diet) AT diet for 4 mos post-weaning. Lung AT concentrations in the 3 groups of either sexes were 0.61±0.14, 17.51±2.68, and 43.78±5.1 (nmoles/g wet wt), respectively. Lung mRNA profiles from basic or high AT fed mice with those from mice fed the minimal AT showed 150-600 differentially expressed genes. A set of 13 genes became the focus of further analysis because all of them appeared to have cytoskeleton functions. These genes are members of the myosin, troponin and tropomyosin families, alpha actin, keratin complexes I and II, repetin and loricrin. The AT related induction of these genes was confirmed by RT PCR. Immuno-histochemical analyses suggest that some of these genes are expressed in lung vasculature and alveolar septal regions. Bioinformatic analyses of these genes suggest that their expression is regulated by serum response factor, a transcription factor required for development and differentiation of cardiac and smooth muscle. Hence, results suggest that AT may play an important role in the cytoskeleton architecture of lung cells.

Development and characteristics of high-fat-diet-induced obesity in Sprague-Dawley rats

Taesun Park, Yun Jung Kim, Haemi Lee, and Hyenam Lee

Department of Food and Nutrition, Yonsei University, Seoul, Republic of Korea

The following experiment was undertaken to establish a model for a rat with diet-induced obesity, presenting a body weight gain along with distinctive characteristics of human obesity and metabolic syndrome that are more profound than those that have been reported previously. Sprague-Dawley rats were fed either the high-fat diet (HFD) (AIN-76A-based, 17 g lard + 3% corn oil/100 g diet) or the normal diet (ND) (AIN-76A-based, 5 g corn oil/100 g diet) for 9 wks. The HFD rats weighed 55% more than did the ND rats, and accumulated significantly greater visceral fat (85-133% greater depending on the site). The HFD rats acquired dyslipidemia, fatty liver, insulin resistance, and hyperleptinemia, typically associated with human obesity. The expression of leptin, TNFα, and resistin genes in the epididymal adipose tissues, which are involved in insulin resistance and hyperleptimenia, were upregulated 7.0-, 3.4-, and 1.5-fold, respectively, by the HFD. The overexpression of several adipocyte transcription factors involved in adipogenesis and adipocyte differentiation, such as PPARγ2 (2.6-fold), C/EBPδ (2.5-fold), and SREBP1c (5.1-fold), in the epididymal adipose tissues of the HFD rats, corresponds to the marked visceral adiposity observed in this animal model. Therefore, diet-induced obesity was developed in the rats that had been fed the HFD within 9 wks, with an almost 200% body weight gain. Since this obesity model is well controlled and has many features similar to those of human obesity, it can therefore be used as a model for the study of the physiologic and molecular abnormalities of high-fat-diet-induced obesity.

Renin Angiotensin System (RAS) inhibition attenuates mitochondrial dysfunction, oxidant production and cardiac hypertrophy in spontaneously hypertensive rats (SHR)

Barbara Piotrkowski, Elena M.V. de Cavanagh, Osvaldo R. Koch[1] and César G. Fraga

Physical Chemistry-PRALIB, School of Pharmacy and Biochemistry, and [1]School of Medicine, University of Buenos Aires

In previous work, we showed that RAS inhibition prevented the decay of mitochondrial function in kidneys of SHR, independently of blood pressure (BP) reduction. Here we studied whether a non-BP lowering dose of enalapril, an angiotensin converting enzyme inhibitor, could protect cardiac tissue and mitochondria from hypertension-related dysfunction. Three-month-old SHR received water containing enalapril (10 mg/kg/day, E) or no additions (S) for 5 months. Wistar-Kyoto rats (W) were normotensive controls. At the end of the study, BP was higher in E and S relative to W. In S, heart/body weight ratio was higher than in E and W. In S, but not in E and W, myocardiocytes were replaced by fibrotic tissue. Matrix metalloprotease activity was lower in E with respect to S and W. In S, mtNOS activity and eNOS expression and activity were lower compared to E and W. Mitochondrial membrane potential in E was lower than in S and W; and H_2O_2 production was higher in E and S compared to W. In S, Mn-SOD activity was higher than in E and W. NADH-dehydrogenase activity was lower in S and E than in W. In summary, in SHR enalapril protects from cardiac hypertrophy, and attenuates several parameters of cardiac mitochondria dysfunction, independently of its known effects on BP.

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Angiogenesis in mouse NZO and NZO/SJL resembles the human metabolic syndrome X

A. Polus, U. Razny, L. Wator, J. Stachura*, G. Dyduch*, G. Joost **, A. Dembiñska-Kiec

*Department of Clinical Biochemistry & *Department of Pathology, Jagiellonian University, Medical College, Krakow, Poland; **German Institute of Human Nutrition, Potsdam-Rehbrucke, Germany*

Obesity, insulin intolerance, hypertension, dyslipidemia and hyperleptinemia are the main clinical symptoms of metabolic syndrome leading to micro- and macrovascular injury, atherosclerosis, diabetes and pathological angiogenesis. Aim of the study was to define the possible link between metabolic syndrome biochemical parameters and angiogenesis. Two mice models with some parameters of human metabolic syndrome X were used NZO and NZO/SJL (kindly obtained from G. Joost Potsdam). Mice were fed with standard and high fat diet for seven weeks. During that time they were weighted and biochemical parameters in serum (glucose, triglycerides and cholesterol also leptin and adiponectin) were measured. During last week of feeding animals were injected subcutaneously with matrigel plugs. After six days matrigel plugs were excised and immunohistochemistry using anti-CD31 (PECAM) antibody was performed.

Results: The feeding with the high fat diet increased body weight, significantly increased concentration of glucose, cholesterol and leptin in serum. In the contrast, serum triglicerides did not differ significantly. Some tendency to decrease adiponectin concentration in serum was observed. Immunohistochemical analysis of angiogenesis parameters in matrigel sections has shown tendency to rise number vessels with and without lumen or CD31 (PECAM) -positive cells in matrigel by high fat diet.

Conclusion: High fat diet promotes symptomes characteristic for human metabolic syndrome in in NZO mice and paralelly activates angiogenesis measured by matrigele implantation model.

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Markov logic networks for aging process modeling

Steve Racunas and Pat Langley

Stanford University, Stanford, CA, USA

We are developing software tools for computer-aided hypothesis design and evaluation. These tools are being designed specifically for biological hypotheses. We would like these tools to take advantage of the information stored in biomedical knowledge bases and we would like to use them to support and test hypotheses about key biochemical processes. In previous work, we deployed our prototype Hypothesis-Space Browser software ("HyBrow") on a Galactose metabolism test system. We then developed methods to proofread knowledge bases of metabolic processes for internal consistency, finding and removing curational errors or artifacts that would cause problems for an automated reasoner such as HyBrow. In our current work, we show how to extend the Hypothesis Browser framework to accommodate a broader class of hypotheses: those expressed using weighted (full) first order logic representations. We demonstrate that the entire Reactome knowledge base can be stored in our extended framework and used to supply supporting or contraindicating evidence for complex hypotheses, and we show that the entire Furber aging model can be phrased in the extended hypothesis language.

Regulation of CuZn-SOD and Mn-SOD mRNA expression by spatial variations in shear stress

Mahsa Rouhanizadeh and Tzung K. Hsiai

University of Southern California, Department of Biomedical Engineering and Cardiovascular Medicine, Los Angeles, CA 90089, USA

Spatial variations in shear stress play an important role in the focal and eccentric characteristics of atherosclerotic lesions. Within the vascular bifurcations, the medial wall, where pulsatile shear stress (PSS) is known to develop, is protective from atherosclerosis. Conversely, the lateral wall, where oscillatory shear stress (OSS) occurs, is prone to atherogenesis. We tested whether PSS vs. OSS differentially regulates vascular oxidative stress via SOD isomers; namely, SOD1 (CuZn-SOD), SOD2 (Mn-SOD. Confluent bovine aortic endothelial cell (BAEC) monolayers and bovine smooth muscle cells (VSMC) were exposed to PSS and OSS in a parallel plate flow system for 4 hours. Total RNA was isolated using the RNeasy kit (Qiagen) and was reverse-transcribed using the SuperScript III Platinum Two-Step qRT-PCR Kit with SYBR Green (Invitrogen), followed by PCR amplification and detection. For quantification of relative gene expression, the target sequence was normalized to 18s rRNA. BAEC SOD mRNA expression: Endothelial SOD2 was significantly up-regulated in response to shear stress. PSS induced a greater up-regulation (10.48-fold, $P < 0.05$, n=3) than did OSS (5.21-fold, $P < 0.05$). SOD1 was significantly increased by 2.29-folds ($P < 0.05$) in response to PSS, but remained unchanged in response to OSS. SMC SOD mRNA expression: Both smooth muscle SOD1 and -2 were up-regulated in response to PSS and down-regulated in response to OSS. However, SOD2 expression was statistically significant [PSS: 1.81-fold; OSS: 0.78-fold; ($P < 0.05$), n=3]. Shear stress increased expression of SOD1 and -2 suggesting that spatial variations in shear stress may contribute to spatial differences in vascular oxidative stress.

Investigation of anti-inflammatory activity of complex herbal oil extract *in vitro* and *in vivo*

Rydlovskaya A., Makarov V., Makarova M., Tihonov V.

Interregional Center "Adaptogen", St-Petersburg, Russia

Known from ancient China and India by their therapeutic qualities Boswellia serrata and Curcuma longa now is an object of study of many scientists. Turmeric extract shows strong antioxidant activity and can inhibit COX activity [1]. Boswellic components inhibit 5-LOX activity [2] and NF-kB transcription factor [3]. As a result two perspective therapeutic nature substances work by different mechanisms of action and in sum can show synergistic effect. It served as basis for their unification and creating of complex preparation (code name: BsCl). Testing of created composition confirmed correctness of choice. By using of mononuclear cells of healthy donor's blood BsCl showed ability to inhibit LPS-inducible production of pro-inflammatory cytokines (TNFα, IL-1β) and metabolites of arachidonic acid (PGE_2, LTB_4). On the basis of TBA-reactive products concentration decreasing in presence of BsCl and results of measuring of its antiradical activity in relation to $HO^{\cdot}$ we could add antioxidant properties to the list of BsCl potentialities. As a result, possibility to show anti-inflammatory action by several mechanisms simultaneously stands out BsCl from the rank of existing synthetic therapeutic remedies. We got conformation of it by testing of the preparation in vivo. By using of model of adjuvant arthritis on Wistar rats BsCl showed anti-inflammatory action comparable by force with voltaren and prednisalon: 300 mg/kg of BsCl decreased edema, temperature and ulcer formation on infected rat paw, normalized biochemical indexes and decreased TNFα level in blood plasma to zero.

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Variations of total and active myeloperoxidase plasma concentration in endurance horses after a 160 km race

D. Serteyn, T. Art, S. Kohnen, G. Deby-Dupont, and T. Franck

University of Liège, Sart Tilman, 4000 Liège, Belgium

Our aim was to assess if prolonged exercice produced in endurance horses a neutrophil degranulation by measuring the total plasma concentration of MPO by ELISA (Biocode-Hycel, Belgium) and the active fraction of MPO in plasma by the SIEFED technique. In 6 horses, blood was sampled before and after a 160 km race (European Championship, Compiegne 2006). White blood cells, creatine phosphokinase (CPK), total and active MPO contents were determined at T0 and T60. Mean speed of the 6 horses was 16.0 ± 0.7 km/h (267 m/min). Performing the endurance race induced a dramatic significant ($p<0.01$) increase in relative and absolute neutrophils number (T0: 7.34 ± 0.68; T60: 16.51 ± 0.82 109 cells/ml), in CPK levels (T0: 362 +/- 55.6 , T60: 2555 ± 280 IU/l) and in total plasma MPO (T0: 199.2 ± 25.8; T60: 563 ± 62.6 ng/ml). MPO level was significantly correlated with CPK level ($R2 = 0.586$, $p<0.01$) and with total blood neutrophil count ($R2=0.776$, $P<0.01$). A slight increase was also observed for the mean active MPO (T0: 0.94 ± 0.18; T60: 4.3 ± 1.44 mU/ml). Correlation between the post-race values of total and active MPO was not significant. Individual variations of active MPO were observed: 2 horses showed a marked increase, until 8 to10 times the initial values, 2 horses showed a moderate increase, and active MPO in plasma did not vary in 2 horses. Prolonged exercise induced in all the competitors an activation of blood neutrophils, with degranulation and an important release of total MPO. The enzyme remained active in blood in 4 horses sometimes at a high level.

Age-related loss of Akt activity is improved by α-lipoic acid through an insulin-independent pathway

Kate Petersen Shay and Tory M. Hagen

Linus Pauling Institute, Oregon State University, Corvallis, OR, USA

Lipoic acid (LA), a dithiol compound with antioxidant properties, is effective against age-related increases in oxidative stress in post-mitotic tissues. LA is rapidly cleared from tissues and the plasma, so the mode of action for its longer term effects on stress resistance is poorly understood. LA has been used to improve glucose utilization through insulin receptor (IR) pathway-mediated Akt phosphorylation, and therefore it may be able to activate signal transduction. Akt is also involved in stress response and cell survival. Based on the known heightened cellular susceptibility to chronic stress with age, we hypothesized that there is an age-related lesion in Akt activity via loss of phosphorylation; furthermore, treatment with LA should reverse this lesion. We showed than in hepatocytes from old rats, the basal level of phospho-Ser473 Akt is 30-40% lower when compared to young, but the basal level of phospho-Thr308 Akt is unchanged. Thr308 is phosphorylated through the IR pathway, indicating that the impaired Akt Ser473 phosphorylation with age was not due to a lesion in the IR pathway. Treatment with physiologically relevant doses of LA (50-100μM) provided a 30% increase in phospho-Ser473 after 30-60 minutes. To test whether LA is acting through the IR pathway to ameliorate age-related loss of Akt phosphorylation, hepatocytes were cultured in insulin-free media until the level of phospho-Akt was undetectable, and insulin or LA was added to the cells. While insulin-treated cells showed a robust response in Akt phosphorylation within 5 minutes, LA-treated cells still required 30-60 minutes to provide a comparable response. These results suggest that at least part the effect of LA on Akt in aged hepatocytes is independent of the IR pathway.

Characterization of antioxidant principle from methanol extract / fractions of Acacia nilotica willd. ex del

Rajbir Singh[a], Bikram Singh[b] and Saroj Arora[a]

[a]Department of Botanical & Environmental Sciences, Guru Nanak Dev University, Amritsar- 143005, [b]Division of Natural Plant Products, IHBT, Palampur, India

Plant produce antioxidant compounds that bind metal ions, scavenge reactive oxygen species and break chains of oxidation. Therefore the plant-based dietary antioxidants are believed to have an important role in the maintenance of human health because our endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species (ROS). With this objective in view, present study was planned to explore the protective effects of methanol extract/fractions of Acacia nilotica Willd. Ex Del. using in vitro experiments, e.g., DPPH, hydroxyl radical (site specific and non site specific), relative reducing power, chelating power and lipid peroxidation assay. The different extracts of this plant were prepared by soaking the fine bark powdered material in respective solvents by increasing as well as decreasing order of solvent polarity. In all these antioxidant-testing assays water fraction showed more prominent effects (94.69%, 78.57%, 64.94%, 1.972, 97.38%, 95.59%) as compared to the crude extract and its ethyl acetate fraction. The inhibitory effect of extracts/fractions was compared with standard polyphenols (L-ascorbic acid and BHT). To identify the antioxidant principle, the methanol extract was subjected to column chromatography and different polyphenolic compounds are isolated. Further studies are in progress to characterize the isolated compounds by NMR, LC-Mass, TLC and other spectroscopic techniques.

Differentiation between central nervous and systemic reactive oxygen species in endotoxemic shock

Sommer, O.*, Fink, B.**, von Dobschuetz, E.*

**Department of General and Visceral Surgery, University of Freiburg, Germany; **Noxygen Science Transfer & Diagnostics GmbH, Germany*

Severe septic shock is often associated with impairment of brain function. Unknown is about the effects of endotoxemic shock on central nervous damage caused by the local generation of reactive oxygen species (ROS) due to the lack of a suitable method to differentiate between local central nervous and systemic release. Therefore the aim of our investigation was to differentiate in vivo between the formation of ROS in central nervous system and systemic release by combination of a microdialysis probe and a venous catheter. ROS were detected using BenchTop EPR and spin probe CMH. Stereotactic implantation of the microdialysis probe (CMA® 12) was performed according to coordinates of Paxinos and Watson or Paxinos and Franklin. Primarily CMH was infused via the microdialysis probe (5μl/min). After 2h the rats were killed and blood samples were taken immediately. In both brain areas high radical concentrations and in peripheral blood no paramagnetic signal could be detected. Reverse results with systemic ROS and no brain radical formation were detected when CMH was applied via a venous catheter and the microdialysis probe flashed with ringer. Mouse experiments were 3h with a 90 min control period in rats and 1,3 μl/min in mice, followed of a LPS (100μg/kg) period of 210min. LPS enhances radical formation up to 35±7% (n = 4) and was not detectable in blood when CMH was infused via the probe. LPS treatment increases peripheral ROS up to 26 ±4% (n = 5) after venous CMH application. The method used is suitable to differentiate between central nervous and systemic released ROS. Although the blood brain barrier seems to be intact there is a local increase of ROS in central nervous system after LPS infusion which might be the mechanism of brain damage in septic shock.

Proteomics analysis reveals a preferential age-related loss in valosin-containing protein in the hepatic endoplasmic reticulum

Jung H. Suh, Robert Kim, Brian M. Dixon, Tory. M. Hagen, and Bruce N. Ames

Children's Hospital Oakland Research Institute, Oakland, CA, USA

Age-related changes in the protein expression profile of the endoplasmic reticulum have received little attention. We compared the protein expression profiles in the endoplasmic reticulum (ER) fractions isolated from young (3-4 mo; N=9) and old (26-28 mo; N=8) rat livers, using 2D gel electrophoresis and mass spectrometry. Out of 387 total spots detected, 31 proteins differed by more than 2-fold, with only 7 proteins increasing in the aged group. Out of the remaining 24 proteins that decreased with age, the lower abundance of the 97 kDa, Valosin-Containing Protein (VCP) was deemed to be most significant based on the magnitude of its decrease and the relative importance of its functions in DNA repair, membrane fusion/biosynthesis and the retrograde transport of misfolded proteins for degradation. VCP is well-conserved across species and abundantly expressed in most mammalian cells, especially in the ER. Western blot analysis, using a specific monoclonal antibody, confirmed that hepatic ER VCP levels decreased by 63±5% ($p=0.007$) in old rats. The total hepatic VCP protein and mRNA levels were also measured and were 21% ($p=0.023$) and 41% ($p=0.01$) lower in old rats, respectively. Phosphorylation of VCP on serine-784 may serve as an ER targeting and/or retention signal. To this end, total hepatic phospho-VCP levels in the ER were also found to be markedly lower in the aging rat liver. Furthermore, caloric restriction attenuated the loss of both total and phosphorylated VCP levels in the aging rat liver. Our results suggest that the preferential loss of ER VCP is due to alterations in its transcription, translation, and/or post-translational regulation that may have significant adverse affects on ER and cell's adaptive stress responses.

Chromatin immunoprecipitation assay links Nrf2-ARE and AP1 in the basal transcriptional regulation of glutamate cysteine ligase catalytic subunit

Swapna V. Shenvi, Sara Haidar, and Tory M. Hagen

Linus Pauling Institute, and Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA

Glutathione (GSH), a low molecular weight antioxidant, declines under chronic inflammation, leading to loss of stress resistance. In humans, GSH levels are, in part, regulated by Nrf2, a transcription factor (TF) binding to the antioxidant response element (ARE) present in the 5' UTR of glutamate cysteine ligase (GCLC), the rate-limiting enzyme involved in its synthesis. However, whether it is the 3 AREs or the AP1 promoters that govern the basal transcription of hepatic GCLC in rats, is currently controversial. To evaluate TF binding to these promoters in an endogenous chromatin configuration, we performed chromatin immunoprecipitations (ChIPs) on cultured rat hepatocytes using antibodies to Nrf2, its binding partners and AP1 TFs. Results show that Nrf2 only binds to the ARE located -3844 bp relative to the start site with mafK and CBP as the major co-activators. Interestingly, this ARE has an embedded AP1 site that binds c-jun and c-Fos simultaneously with Nrf2. One way of inducing Nrf2-mediated GCLC expression is through the dithiol R-(α)-lipoic acid (LA). In order to determine whether the inducible transcription of GCLC is similar to the basal machinery, we treated hepatocytes with 100 μM LA and performed ChIPs. Results show a 67% increase in Nrf2 after 1 hr that is maintained for 24 hrs, but no significant change in mafK or CBP. Concomitantly, jun-Fos declined by 75% after an hr, but c-jun binding was restored to baseline after 24 hrs. The heightened Nrf2 binding corresponded to a 40% increase in GCL activity and a 20% increase in GSH levels. Taken together, these results suggest that LA may upregulate hepatic GSH levels by increasing the basal transcription of GCLC through Nrf2-ARE.

Sesaminol glycosides provide a neuroprotective effect against β-amyloid peptide induced oxidative stress in SK-N-SH human neuroblastoma cells

Min Young Um, Ji Yun Ahn, Sung Ran Kim, Tae Youl Ha

Food Function Research Division, Korea Food Research Institute, Seong Nam, Gyeonggi-Do, Korea

Oxidative stress plays a significant role in Alzheimer's disease (AD). The neurotoxic β-amyloid (Aβ) contributes to oxidative damage in AD by inducing lipid peroxidation, which in turn generates additional downstream cytosolic free radicals and reactive oxygen species (ROS), leading to ultimate apoptosis. Therefore, attenuation of oxidative stress by timely antioxidant application is proposed as a potential therapeutic intervention in AD. Sesaminol glucosides (SG) is one of the lignans found in the sesame and showed inhibitory effects on susceptibility to oxidative stress in hypercholesterolemia rabbit. In this study, we have investigated the neuroprotective effects of sesaminol glucosides on Aβinduced oxidative cell death in SK-N-SH human neuroblastoma cells. Following exposure of the SK-N-SH cells to Aβ for 24 hours, a marked neuronal injury, increases of intercellular malondialdehyde (MDA) and reactive oxygen species (ROS), and imbalance of antioxidant enzymes activities were observed. Aβ treatment also upregulated apoptosis related protein expression. However, SG treatment inhibited Aβ induced cell death by preventing intracellular ROS accumulation, imbalance of antioxidant enzymes activities, and apoptosis, as well as by suppressing stress-activated kinase c-Jun N-terminal kinase (SAPK/JNK-P) and extracelullar signal-regulated kinase (MAPK/ERK-P). The results suggest that SG has a protective effect against Aβ-induced neuronal apoptosis possibly through scavenging free radicals.

Cigarette smoke modulates scavenger receptor B-1 expression and localization in airway epithelial cells

G. Valacchi, P.A. Davis, E.M. Khan, S.S. Castillo, M. Levy, R. Lanir, C.E. Cross and T. Goldkorn

CCRBM, University of California Davis, Davis, California, USA

Scavenger Receptor B1 (SR-B1) has been shown to play a prominent role in the uptake and delivery of vitamin E (α-tocopherol) from HDL to the cells regulating the vitamin E status of the lung. However, the mechanism (s) of the lung's delivery systems for tocopherol and other antioxidants remain incompletely understood. Our in vivo studies demonstrated that lung SR-B1 expression levels (protein and RNA) can be modulated by cigarette smoke (CS) exposure. To further characterize the molecular mechanism (s) of SR-B1 modulation under CS we exposed human airway epithelail cells A549 to various doses and time points of CS. Subsequently, we demonstrated by both FACS analyses and confocal microscopy that SR-B1 colocalizes mainly to the perinuclear area in control cells. However, in the CS exposed cells SR-B1 was translocated to the cell surface membrane and localized in large patches. This localization was lost after 24 hours, with a drammatical decline in SR-B1 protein expression. Finally the effect of CS on SR-B1 mRNA stability was, mRNA was extracted at 0, 1, 2 , 3 hrs after CS exposure and SR-B1 messenger level determined by RT-PCR. Values declined similarly between the air and the CS treated cells, indicating that CS had no signficant effect on mRNA stability. These results not only parallel 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 and possible selective trafficking, could modulate tocopherol transport in response to CS (as well as other oxidant assaults) in lung epithelial cells.

Membrane potential modulates mtNOS activity and NO diffusion to the cytosol

Laura B. Valdez, Tamara Zaobornyj and Alberto Boveris

Laboratory of Free Radical Biology, School of Pharmacy and Biochemistry, University of Buenos Aires. Buenos Aires, Argentina

Mitochondrial metabolic state regulates the rate of NO production from heart, liver and kidney mitochondria. NO release by heart mitochondria was about 45% lower in state 3 (1.22 ± 0.05 nmol/min.mg protein) than in state 4 (2.23 ± 0.06 nmol/min.mg protein). In the state 4-state 3 transition the activity of mtNOS, responsible for NO release, is driven by the membrane potential and not by intramitochondrial pH changes. NO release by rat liver mitochondria showed an exponential dependence on membrane potential: agents that decrease or abolish membrane potential, minimize NO release; while the addition of oligomycin, that produces mitochondrial hyperpolarization, generates the maximal NO release. A similar behavior was reported for heart mitochondrial H_2O_2 production (Korshunov et al., 1997). The fraction of cytosolic NO provided by diffusion from mitochondria was 61% in heart, 47% in liver, and 30% in kidney. The intramitochondrial concentrations of L-arginine and NADPH are higher than their KM values, and the changes in their concentrations in the state 4-state 3 transition are not enough to explain the changes in NO release. These data indicate that the redox state of the respiratory chain components regulates H_2O_2 production and $\Delta\Psi$ modulates NO release, and support the speculation that NO and H_2O_2 report a high mitochondrial energy charge to the cytosol. The regulation of mtNOS activity, an apparently voltage-dependent enzyme, at the physiological range of membrane potentials, makes mtNOS a regulable enzyme that in turn regulates mitochondrial O_2 uptake and H_2O_2 production.

Champagne wine polyphenols protect primary cultured neuronal cells against peroxynitrite-induced toxicity

David Vauzour, , Melanie Pruden, , Katerina Vafeiadou, and Jeremy P.E Spencer

The University of Reading

White wines are generally low in polyphenol content, in particular flavonoids, compared to red wines. However, champagne wines have been shown to contain relatively high amounts of phenolics such as tyrosol and caffeic acid. Such phenolics have not been thoroughly investigated for their ability to modulate cell injury induced by oxidative stress. In this study, we have investigated the potential protective effects of champagne wine extracts and individual components and their metabolites to protect against peroxynitrite-induced neuronal injury. The CWE was found not to be neurotoxic at concentrations up to 50 μg/ml and exhibited potent neuroprotective activity against peroxynitrite-induced injury at low concentrations (0.1 μg/ml). Tyrosol, caffeic acid, gallic acid and homovanillyl alcohol were also observed to exert potent neuroprotection at concentrations between 1 and 50 μM. However, at concentrations above 50 μM a smaller level of protection was observed. In order to determine a mechanism of action for the observed neuroprotection, we investigated whether individual polyphenols present in the CWE induced effects on specific intracellular signalling pathways important in determining neuronal survival. The activation of both Akt/PKB and ERK1/2 by polyphenols paralleled protective effects in that concentrations ranging from 1-50 μM induced significant increases in phosphorylation above basal levels, whereas at higher concentrations the activation was less marked. Together these data suggest that polyphenols present in champagne wine may induce a neuroprotective effects possibly by interacting with neuronal survival signalling pathways.

Mild carbon monoxide exposure causes oxidative stress

D. Webber, D. Acuna, I. Lopez, and J. Edmond

University of California Los Angeles, Los Angeles, California, USA

Air contains carbon monoxide (CO) from tobacco smoke and the burning of carbon based fuels. We find that mild chronic CO exposure creates oxidative stress that impairs neurons in the spiral ganglion, and in other cochlear regions. We examined the effect of chronic mild CO exposure (25 ppm) and iron availability on auditory development. CO exposed rat pups have decreased neurofilament proteins and increased copper, zinc-superoxide dismutase (SOD1) in the neurons of the spiral ganglion. The increase in the SOD1 enzyme causes increased production of hydrogen peroxide, thus favoring the Fenton reaction to initiate oxidative injury. But the CO exposed group with decreased iron availability exhibited an up-regulation of transferrin in their cochlea, and the expression of neurofilament proteins and of SOD1 were similar to controls. The normal expression of SOD1 and reduced iron availability does not initiate oxidative injury. We conclude that the developing cochlea is selectively affected by mild CO exposure causing an increase in oxidative stress, while limiting iron availability ameliorates the effect caused by mild CO exposure by averting conditions that facilitate oxidative stress. Oxidative stress is known to be involved in the initiation of cardiovascular disease and is a component of many neurodegenerative diseases. Tobacco smoke is known to aggravate the condition of patients with ALS and MS. Consequently, we suggest that chronic mild CO exposure from tobacco smoke and/or from the incomplete combustion of fossil fuels promotes oxidative stress and thus exacerbates the oxidative injury in these conditions.

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Aging up-regulates expression of inflammatory mediators in mouse adipose tissue (AT)

Dayong Wu, Munkyong Pae, Zhihong Ren, Weimin Guo, Xuelin Cui, and Simin Nikbin Meydani

JM USDA HNRCA at Tufts University, 711 Washington Street, Boston, MA 02111

The incidence of type 2 diabetes (T2D) increases with age. Low-grade inflammation in AT is implicated in development of insulin resistance and T2D. This study investigated if inflammatory responses are up-regulated with age in AT. Results showed that visceral AT from old mice have higher mRNA expression of IL-1β, IL-6, TNF-α, and COX-2 (263, 208, 165, and 115% higher, respectively) and lower (60%) PPAR-γ than those of young. Adipocytes (AD), and not stromal vascular cells including Mϕ, are the cells responsible for this age-related difference, which is thus distinguished from obesity-related AT inflammation in which Mϕ are the main contributor. Mϕ of either age (young or old) produced more IL-6 in response to old AD-conditioned medium (CM) compared to young CM. This suggests that in addition to producing more IL-6, AD from old mice can induce more IL-6 by other cells such as Mϕ. Addition of ceramide or sphingomyelinase increased IL-6 production in young AD to a level comparable to that seen in old AD. Inhibiting sphingomyelinase, ceramide synthesis, or NF-κB activation reduced IL-6 production by AD. NF-κB regulates expression of several inflammatory products and ceramide was shown to induce NF-κB activation. Thus, these data suggest a potential role for ceramide and NF-κB in the age-related increase of AT inflammation. Further study is needed to determine the underlying mechanisms of the observed effects and their contribution to T2D in the aged.

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Ovarian hormone neuroprotection against mitochondrial toxins

Jia Yao, Jon Nilsen, Diana Perez, Ashkon Ansari, and Roberta Díaz Brinton

Department of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033, USA

We have investigated the role of mitochondria in neuroprotection induced by ovarian hormone therapy and shown that the neuroprotective effects of 17β-estradiol (E2) are dependent upon intact mitochondrial function and are coupled with increased mitochondrial calcium sequestration. Further, many of the signaling cascades induced by E2 treatment converge upon the mitochondria. In current study we sought to determine the neuroprotective efficacy of ovarian hormones against mitochondrial toxins in an initial attempt to identify the mitochondrial site of action of these compounds. We performed dose response neurotoxicity studies in primary hippocampal neuron cultures to identify the appropriate dose for neuroprotective experiments. Mitochondrial toxins employed were rotenone (0.01 μM-1 μM), KCN (100 μM-10 mM), methylmalonate (0.1 μM-1 mM), amiodarone (0.01 μM-100 μM), MPP^+ (100 μM-10 mM), antimycin (100μM-500μM), 3-nitropropionic acid (1 mM-20 mM) and oligomycin (0.05 μM-5 μM). All these toxins induced significant neuronal death within 24 hr. The dose of toxin that induced ~30% neuronal death was selected for assessment of ovarian hormone neuroprotective efficacy. Primary hippocampal neurons were pretreated with varying doses (0.1 ng/mL-1000 ng/mL) of E2 or P4 for 48 hr prior to a 24 hr exposure to the mitochondrial toxins. Short-term treatment was ineffective at protecting against the mitochondrial toxins. In contrast, there was significant neuroprotection by pretreatment with E2 for 7 days prior to the toxin exposure.

Formation of GSNO in astrocytes and neurons Implications for neurotoxicity

Li Peng Yap, Derick Han, and Enrique Cadenas

Molecular Pharmacology and Toxicology, School of Pharmacy
University of Southern California, Los Angeles, CA 90033

S-nitrosoglutathione (GSNO) has been proposed to function as a modulator of the action of NO. GSNO has potentially significant roles in cell signaling and biological process through S-nitrosylation of proteins and modulation of redox status of cells by altering the GSH pool. To investigate GSNO formation under conditions that mimic NO production by iNOS during inflammation, we exposed primary astrocytes and cortical neurons to a long acting nitric oxide donor (DETA-NO), and measured GSH, GSNO and GSSG by HPLC. Exposure of both astrocytes and neurons to DETA-NO showed formation of GSNO and GSSG. Comparison of GSH/GSNO and GSH/GSSG ratios in both astrocytes and neurons showed both ratios decreased with increasing concentrations of DETA-NO. However, decreases in GSH/GSNO and GSH/GSSG ratios were more dramatic in neurons than in astrocytes, thus indicating a difference in rates of GSNO metabolism and GSSG reduction. Subsequently, the activities of GSNO and GSSG reductase were measured and compared. The activity of both the GSNO reductase and GSSG reductase in astrocytes were significantly higher than in neurons. Analysis of GSNO consumption in neurons and astrocytes by HPLC also showed a subsequent increase in the formation of GSSG. Taken together, the data suggest that the glutathione defense system in astrocytes is an efficient defense against nitrosative stress. This is especially important in the context of neurodegenerative disease associated with neuroinflammation where there is selective loss of neurons concomitant with a long lasting activation of iNOS.

Mechanisms of aging: Time to draw resolution

Dazhong Yin

Aging Biochemistry Laboratory, College of Life Sciences,
Hunan Normal University, Changsha 410081, Hunan, China

Although it is gradually accepted that 'senescence is a collective consequence of both inheritance and environment', the side-reactions of energy metabolism, mainly reactions belong to oxidative stress and nonenzymatic glycosylation (glycation), have been found the critical driving forces of aging-related alterations in animals. The free radical/glycation induced carbonyl stress, the key culprit to form crosslinks, has been identified to cause stable cyclic conjugates of mainly protein-based aggregates implying entropy increase during aging. When combining such key aging processes with age pigment biochemistry, a general picture of aging process can be figured out, as the main clues and the final results are available. While focusing on *process* (irreparable alterations) rather than on *causes* (damages), we can then get a clear view of aging mechanisms: that the irreparable damage accumulation of energy metabolism-associated biochemical side-reactions is the essential and profound nature embedded in higher animals' aging process.

Redox regulation of inflammatory responses by TRX family members; MIF/GIF and TRX

Junji Yodoi[1], Akira Mitsui[2], Noriko Kato[1], Aoi Son[1], Tomijiro Hara[1], Akiko (Bizen) Abe[1], Hiroyuki Tamaki[3] and Norihiko Kondo[1]

[1]Department of Biological Responses, Institute for Virus Research, Kyoto University, Kyoto, Japan.: [2]Pharmaceutical Research Laboratories, Ajinomoto Co., Inc. Kawasaki, Japan.: [3]Department of Gastroenterology & Hepatology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Thioredoxin (TRX) family proteins having an active site sequence -C-X-X-C- are known to function as anti- inflammatory and redox-regulatory protein. We first reported that peptide A and B were purified as adult T- cell leukemia-derived factor (ADF) activity proteins produced by human T-cell leukemia virus type-I transformed ATL2 cells. The peptide A sequence was cloned and reported as ADF/TRX1. Furthermore, we demonstrated that the peptide B was macrophage migration inhibitory factor (MIF)/ glycosylation inhibiting factor (GIF). MIF/GIF is having pro- inflammatory and immune regulatory actions. Recently, we suggested that expression of TRX and MIF/GIF were reciprocally regulated and TRX was associated with inhibition of inflammatory response by suppression of MIF/GIF production. Overexpression of intracellular TRX suppressed MIF/GIF expression in T-cell line and TRX expression was accelerated in CD4+ T cells derived from MIF-deficient mice. It was shown that TRX inhibited MIF/GIF production in patients with inflammatory bowel disease and ameliorated murine dextran sulfate sodium induced colitis (submitted data). It was also shown that TRX attenuated systemic inflammation against cigarette smoking through the suppression of MIF/GIF expression (submitted data). In this report, we discuss the possibility that the anti-inflammatory functions of TRX1 can be explained by the directly interaction with MIF/GIF, thereby masking or silencing the action of MIF pro-inflammatory activity.

Effect of cholesterol depletion on cytotoxicity of *Vibrio vulnificus* cytolysin on human neutrophils: the role of plasma membrane cholesterol in *V. vulnificus* cytolysin-mediated cellular function

Hong-Nu Yu[1], Young-Rae Lee[1], Eun-Kyung Song[2], Myung-Kwan Han[2], Sang-Young Lee[3], and Jong-Suk Kim[1]

[1]Department of Biochemistry, [2]Microbiology & Immunology, [3]Radiology, Chonbuk National University Medical School, Jeonju, Jeonbuk 561-180, South Korea

In a previous study, we proposed indirect evidence that cholesterol might be a target for *Vibrio vulnificus* cytolysin (VVC) showing that VVC is oligomerized by exogenous cholesterol and subsequently inactivated through the oligomerization. However, it has not been known whether cell associated cholesterol is a real target for VVC. Here, we show that host cell associated-cholesterol is a receptor for VVC. We depleted cholesterol on neutrophil by methyl-β-cyclodextrin (MβCD) extraction and analyzed the effect of cholesterol depletion on cellular toxicity of VVC. VVC rapidly bound to normal neutrophils, but did not bind to cholesterol depleted-neutrophils. Normal neutrophil showed cytotoxicity induced by VVC in a dose-dependent manner whereas cholesterol depleted neutrophil showed the significant inhibition of VVC-induced neutrophil cytotoxicity. In addition, cholesterol depletion inhibited VVC-induced decrease of cellular ATP, potassium efflux, elevation of the cytosolic free Ca^{2+}, and generation of reactive oxygen species (ROS), which are causing mechanisms of cell death. Moreover, cholesterol depletion suppressed VVC-induced pore formation, as evidenced by efflux of 2-deoxy-D-[^{3}H]glucose. These findings suggest that plasma membrane cholesterol is an essential for cytotoxicity of VVC on human neutrophils.

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Induction of phase II/detoxification enzymes by electrophiles in human bronchial epithelial cells

Hongqiao Zhang, Honglei Liu, Alessandra Rinna,
and Henry Jay Forman

School of Natural Science, University of California, Merced, CA95343

Many phase II enzymes are inducible in response to oxidants and electrophiles and this induction is mediated through an electrophile response element (EpRE) in the promoter of these genes. Some studies suggested that the signaling pathways involved in the induction of EpRE-mediated phase II genes are varied, based on experiments performed in different cell types and with different inducers. Whether it is the case in the same cells with the same inducer is unclear; moreover, if it is the case, the underlying mechanism needs to be clarified. This study is designed to answer above questions by using human bronchial epithelial cells (HBE1 cells) as a model. Two electrophiles, 4-hydroxynonenal (HNE) and acrolein activated the major three MAPK pathways (i.e., ERK1/2, p38MAPK, and JNK) in HBE1 cells. Using real-time PCR assay, we found that either HNE (15 μM) or acrolein (5 μM) could induce the expression of phase II genes (GCLC, GCLM, NQO1, NQO2, and HO-1). We then determined the possible MAPK pathways involved by using specific inhibitors to ERK, JNK, and p38MAPK. JNK pathway was involved in the induction of GCLC and GCLM, but not NQO1, NQO2 and HO-1 in HBE1 cells. ERK1/2 and p38, which were found to be involved in EpRE-mediated phase II detoxification gene induction by HNE in other cells, were not involved in HBE1 cells. In conclusion, our data suggest that JNK is involved in Electrophile-mediated GCL induction in HBE1 cells and signaling pathways other than ERK, p38MAPK, and JNK are involved in NQO1, NQO2, and HO-1 up regulation in HBE1 cells.

c-Jun N-terminal kinase (JNK) directly targets mitochondria and regulates mitochondrial pyruvate dehydrogenase (PDH) activity in nervous system

Qiongqiong Zhou, Derick Han, and Enrique Cadenas

Department of Molecular Pharmacology & Toxicology, School of Pharmacy, University of Southern California, CA 90033 U.S.A.

c-Jun N-terminal kinase (JNK) is implicated in neurodegenerative diseases: upon activation, JNK can directly be translocated to mitochondria in many types of cells. The purpose of this investigation is to clarify the potential biological role of JNK in regulating mitochondrial activity in the nervous system. Using experimental models of cultured primary rat cortical neurons and isolated rat brain mitochondria, these studies showed that: (i) Both anisomycin, a potent JNK activator, and hydrogen peroxide can induce the rapid activation of JNK and its association with mitochondria in cultured primary cortical neurons. (ii) Mitochondria-associated active JNK can be degraded by Proteinase K, which indicates that active JNK associates with the outer membrane of mitochondria. (iii) The association of active JNK with mitochondria caused an increase of PDH phosphorylation and its activity inhibition. These results indicate that active JNK may function as a connector between the cytosol and mitochondria, regulating mitochondrial metabolism according to cytosolic environment. The studies acquire further significance, because mitochondrial metabolic activity is critical to nervous system function. Abnormally high level of JNK activity in brain may cause severe inhibition of PDH, which may contribute to the development of pathological stage of many neurodegenerative diseases.

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