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Contents

Keynote Lecture

A Metabolic Tune-up to Optimize Health

Bruce N. Ames

Children's Hospital Oakland Research Center/University of California, Berkeley

An optimum intake of micronutrients and metabolites, which varies with age and genetic constitution, would tune up metabolism and give a marked increase in health, particularly for the poor, young, obese, and elderly, at little cost. 1) DNA damage. Deficiency of vitamins B-12, folic acid, B-6, C, or iron or zinc appears to mimic radiation in damaging DNA by causing single- and double-strand breaks, oxidative lesions or both. Half of the population may be deficient in at least one of these micronutrients. 2) The Km concept. Approximately 50 different human genetic diseases that are due to a poorer binding affinity (Km) of the mutant enzyme for its coenzyme can be remedied by feeding high-dose B vitamins, which raise levels of the corresponding coenzyme. Many polymorphisms also result in a lowered affinity of enzyme for coenzyme. 3) Mitochondrial oxidative decay. This decay, which is a major contributor to aging, can be ameliorated by feeding old rats the normal mitochondrial metabolites acetyl carnitine and lipoic acid at high levels. Mitochondrial decay is accelerated by many common micronutrient deficiencies, such as iron, copper, pantothenate, or biotin, and possibly zinc, with oxidant leakage leading to accelerated aging and neural decay.

Session I
Mitochondria, Aging, and Neurodegeneration

Mitochondrial Role in Cell Death Programs and Neurodegeneration

Dale E. Bredesen

Buck Institute, Novato, CA USA

Whether by developmental design, physiological trigger, or pathological insult, cells are capable of activating suicide programs, and recent evidence suggests that multiple such programs exist. These programs feature in neurodegenerative disease states, neoplasia, autoimmune disorders, and a host of other diseases. Mitochondria have proven to play a prominent role in cell death programs and their associated diseases, and the relevant list of biochemical mediators and mechanisms continues to grow. For example, in apoptosis, direct targeting of mitochondria by proteins that contribute to pore formation, such as Bax, leads to the release of multiple cell death factors, such as cytochrome c, Smac/DIABLO, AIF (apoptosis-inducing factor), endonuclease G, and Omi/Htra2. Some of these proteins also appear to play roles in non-apoptotic cell death programs such as paraptosis, excitotoxin-induced neuronal death, and PARP (poly (ADP-ribose) polymerase) -activation cell death. In neurodegenerative diseases, mitochondrial abnormalities have been demonstrated for all of the major syndromes: in Parkinson's disease, mitochondrial complex I abnormalities exist early in the disease, and rotenone creates a phenocopy of the disease. In Huntington's disease, lymphoblasts from patients demonstrate reduced mitochondrial membrane potentials and more rapid depolarization in response to calcium; moreover, these abnormalities appear in animal models prior to symptoms or pathological alterations. In Alzheimer's disease, abnormalities of mitochondrial complex IV have been demonstrated in both platelets and brain. As the roles assumed by mitochondria in these diseases continue to be defined, it is likely that mitochondrial factors will emerge as key therapeutic targets.

Steroid Hormone Regulation of Mitochondrial Function: Implications for Neuron Survival and Neurodegenerative Disease

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Molecular Pharmacology & Toxicology, Neuroscience Program, University of Southern California, Los Angeles, CA, USA

One characteristic of many neural pathologies, including Alzheimer's disease, is disregulation of Ca^{2+} homeostasis and mitochondrial respiratory function. We have shown that E_2 attenuates the rise in bulk free $[Ca^{2+}]i$ induced by excitotoxic glutamate while potentiating the total influx of Ca^{2+} in a manner that is dependent upon functional mitochondria. We propose that estrogen-induced neuroprotection is due to modifications in mitochondrial function that lead to an increased mitochondrial sequestration of $Ca^{2+}$and an increased mitochondrial Ca^{2+} load tolerability. We demonstrated that E_2 treatment results in an increase in $[Ca^{2+}]m$ sequestration that is coupled to a decrease in $[Ca^{2+}]i$ in response to excitotoxic glutamate. This increased mitochondrial calcium load is coupled with an increased Ca^{2+} load tolerability as evidenced by the E_2-mediated protection against Ca^{2+}- induced mitochondrial swelling and loss of respiratory function. We further showed that E_2 prevents the glutamate- and β A-induced reciprocal mitochondrial/cytosolic translocation of cytochrome c and Bax. These data support the hypothesis that estrogen-induced neuroprotection is mediated by an alteration in mitochondrial function that is accompanied by increased sequestration of cytosolic Ca^{2+} and an offset of downstream effects of this increased $[Ca^{2+}]m$ load.

Supported by: *NIA, the Norris Foundation and the Whittier Foundation to RDB and The John D. French Foundation to JN.*

Detecting Mitochondrial Oxidative Stress in Cultured Neurons

David G. Nicholls and Sabino Vesce

Buck Institute for Age Research, Novato, CA, USA

The relationship is investigated between superoxide levels in single cultured rat cerebellar granule neurons exposed continuously to glutamate in low KCl medium and the deregulation of cytoplasmic calcium. Cells that maintain a regulated cytoplasmic free calcium and mitochondrial polarization in the presence of glutamate show no increase in superoxide levels until the onset of deregulation. Rotenone and antimycin A increase superoxide levels with no effect on cytoplasmic free calcium. The potent cell-permeant superoxide dismutase/catalase mimetic manganese tetrakis (N-ethylpyridinium-2yl) porphyrin, MnTE-PyP, abolishes the deregulation-related increase in superoxide but has no effect on deregulation itself. The partial protection afforded by high concentrations of the related Mn(III)tetrakis (4-benzoic acid) porphyrin, MnTBAP can be ascribed to an inhibition of calcium entry via the NMDA receptor. Following deregulation nuclei undergo condensation; this morphological change is not inhibited by MnTE-PyP and cannot account for the increased ethidium fluorescence. Phospholipase A2 inhibitors decrease the deregulation-related increase in superoxide without protecting against deregulation. It is concluded that while pre-existing oxidative stress is established to be of importance in determining cell survival during subsequent glutamate exposure, NMDA receptor activation does not increase superoxide levels until the onset of deregulation.

Two subpopulations of mitochondria in the aging heart display differing degrees of oxidatives stress.

Tory M. Hagen, Jung H. Suh and Regis Moreau

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Cardiac mitochondria are composed of two distinct subpopulations: one beneath the sarcolemma (subsarcolemmal mitochondria: SSM), and another along the myofilaments (interfibrillary mitochondria: IFM). Previous studies suggest a preferential loss of IFM function with age; however, the age-related changes in oxidative stress in these mitochondrial subpopulations have not been examined. To this end, the changes in mitochondrial antioxidant capacity, oxidant output, and oxidative damage to Complex IV in IFM and SSM from young and old rats were studied. Results show no apparent differences in any parameters examined between IFM and SSM from young rats. However, relative to young, only IFM from old rats had a significantly higher rate of oxidant production and a decline in mitochondrial ascorbate levels and GSH redox status. The age-related decline in mitochondrial antioxidant capacity in IFM was accompanied by a marked loss in glutaredoxin and GSSG reductase activities, suggesting a diminished reductive capacity in IFM with age. Moreover, the loss in Complex IV activity was limited to the IFM of old rats, which was accompanied by a 4-fold increase in 4-hydroxynonenal-modified Complex IV. Thus, mitochondrial decay is not uniform and further indicates that myofibrils may be uniquely under oxidative stress in the aging heart.

Oxidative stress and therapeutic intervention

Simon Melov

Buck Institute for Age Research

The endogenous oxidative stress produced by respiring mammalian mitochondria is potentially injurious. Using a mouse model of mitochondrial oxidative stress, the superoxide dismutase 2 nullizygous mouse (SOD2-/-), we have demonstrated a range of specific tissue and cell specific vulnerabilities to free radicals produced by the mitochondria. In conjunction with the SOD2 -/- mouse, we have also evaluated a number of antioxidants for efficacy in preventing the various pathologies that arise due to endogenous oxidative stress. Data will be presented showing targets at the biochemical and gene expression profile level, which are both vulnerable to mitochondrial oxidative stress, and respond to appropriate therapeutic intervention.

Similar gene expression patterns characterize aging and oxidative stress in *Drosophila melanogaster*

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Affymetrix GeneChips were used to measure RNA abundance for ~13,500 Drosophila genes in young, old, and 100% oxygen-stressed flies. Data were analyzed using a novel background correction algorithm and a robust multichip model-based statistical analysis that dramatically increased the ability to identify changes in gene expression. Aging and oxidative stress responses shared the upregulation of purine biosynthesis, heat shock protein, antioxidant, and innate immune response genes. Results were confirmed using Northerns and transgenic reporters. Immune response gene promoters linked to GFP allowed longitudinal assay of gene expression during aging in individual flies. Immune reporter expression in young flies was partially predictive of remaining life span, suggesting their potential as biomonitors of aging.

In addition our latest results on the mechanism of life span extension by Drosophila MnSOD will be presented.

Heme Metabolism and the Mitochondrial Decay of Aging Brain

Hani Atamna

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Background: Heme controls several aspects of cell metabolism by the expression of specific genes and activity of specific proteins. The research on heme suggests a larger role of heme in cell metabolism, in aging, and age-related disorders. Heme may be the common denominator for a large number of the metabolic changes seen in Alzheimer's disease (AD), including: decrease in complex IV (its assembly depends on heme-a), iron accumulation, increase in heme oxygenase (HO-1), bilirubin (one of the products of HO-1), and increased inhibition of the muscarinic acetylcholine receptor by heme or a heme-metabolite.

Objective: We have measured and compared the content of heme (free from hemoglobin), heme-a, the ratio heme-a/heme, and level of ferrochelatase in the temporal lobe of brain of AD patients and in age-matched normal subjects.

Results: The level of heme was 2.3-fold higher, while heme-a was 25 % lower in AD brains. The ratio heme-a/heme was 2.6-fold lower in AD brains. Heme synthesis appears increased in the temporal lobe, as the level of ferrochelatase (a mitochondrial enzyme that inserts Fe^{+2} into heme) was also 4-fold higher in AD brains.

Conclusions: Heme metabolism has changed in AD brain, which may contribute to the mitochondrial decay, oxidative stress, and iron accumulation. The amino acid sequence of amyloid-b contains three histidines that can bind heme. The biochemical consequences of altered heme metabolism and possible future therapy of AD will be discussed.

The Lon Protease, Aging, and Degenerative Diseases

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Lon is a major regulator of multiple mitochondrial functions. It catalyzes the degradation of oxidatively-modified matrix proteins, chaperones the assembly of inner membrane complexes, and participates in the regulation of mitochondrial gene expression and genome integrity. We have found strongly decreased Lon transcription and translation during aging and in certain age-related degenerative diseases, and we wanted to model the effects of decreased Lon levels in a simple cell culture model using Lon ntisense morpholino oligonucleotides. An early result of Lon down-regulation in WI-38 VA-13 human lung fibroblasts is massive caspase 3 activation and extensive (although not universal) apoptotic death. At a later stage, the surviving cells fail to divide, display highly abnormal mitochondrial function and morphology and, rely almost exclusively on anaerobic metabolism. Mitochondrial mass decreases with mitochondrial replication, as a result of mitochondrial inability to divide. Eventually, cells are lost by necrosis or 'mitoptosis.' Our results indicate that mitochondrial Lon is required for normal survival and proliferation, and provide clues to the possible effects of decreased Lon during aging and in age-related degenerative diseases.

Cardiolipin-cytochrome *c* Interaction in the Mitochondrial Regulation of Caspase Activation and Apoptosis

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It is now well established that the mitochondria play an important role in the regulation of apoptotic cell death by mechanisms which have been conserved during evolution. Thus, it seems that a host of lethal agents target the mitochondria and stimulate their release of cytochrome c and other proteins, which can trigger caspase activation and apoptosis. This release is governed by the Bcl-2 family of proteins and occurs by a two-step process, which is initiated by dissociation of the hemoprotein from cardiolipin, the phospholipid that anchors it to the outer surface of the inner mitochondrial membrane. Mounting evidence suggests that a decrease in the level of cardiolipin affects cytochrome c binding to the inner membrane, thus leading to higher levels of soluble cytochrome c in the mitochondrial intermembrane space. Among factors known to affect the cardiolipin level are free fatty acids (16:0,18:0), calcium dysregulation and reactive oxygen species (ROS). These factors, particularly calcium and ROS, have long been recognized as triggers of cell death and, more recently, as modulators of mitochondrially-mediated apoptosis. In this presentation, I'll discuss the significance of the disruption of the cardiolipin-cytochrome c interaction for cytochrome c release and apoptosis.

Session II
Micronutrients: Vitamin E

Metabolism of vitamin E and gene regulatory functions

Brigelius-Flohé R, Bumke-Vogt C, Kluth D,
Landes N, Pfluger P, Müller-Schmehl K

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All forms of vitamin E are metabolized by side chain degradation initiated by an ω -hydroxylation catalyzed by a cytochrome P450 enzyme (CYP). All forms of vitamin E tested so far are degraded to a much higher extent than α-tocopherol. CYPs degrade various endogenous and exogenous compounds and some of them are induced by their substrates themselves. We could recently show[1] that vitamin E induced a reporter gene driven by the pregnane-X-receptor (PXR), which is activated by a high number of structurally diverse compounds. The induction was highest with α- and γ-tocotrienol (T3) and low but significant with α-tocopherol. In addition, γ-T3 increased the mRNA of endogenous CYP3A4 in HepG2 cells. Since these *in vitro* findings imply that vitamin E might interfere with the metabolism of drugs, it deemed necessary to investigate their relevance *in vivo*. To this end, mice were grown with low, normal, and high amounts of α-tocopheryl acetate for three months. In addition, half of each group was supplemented with 250µg γ-T3/day for 7 days. Liver mRNA was prepared and analyzed for transcription of CYP3a11, the murine homolog of human CYP3A4, by real time PCR. CYP3a11 increased with the dosage of α-tocopherol, but not by additional g-T3. An explanation for the low inductive potency of γ-T3 might be the fast metabolization that is evident from the urinary excretion of γ-CEHC, the final degradation product of γ-T3.

[1]Landes N, Pfluger P, Kluth D, Birringer M, Rühl R, Böl G-F, Glatt H, Brigelius-Flohé R (2003) Biochem Pharmacol 65, 269-273

Regulation of Gene Expression by Vitamin E

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Several genes are regulated by tocopherols. They can be grouped in five categories:

Group 1. Genes that are involved in the uptake and degradation of tocopherols: alpha-TTP, Cytochrome P450 (CYP3A), and glutathione-S-transferase .

Group 2. Genes that are implicated with lipid uptake and atherosclerosis: CD36, SR-BI, SR-AI/II.

Group 3. Genes that are implicated with modulation of extracellular proteins: Tropomyosin, Collagen alpha1, MMP-1, MMP-19, CTGF.

Group 4. Genes that are related to inflammation, cell adhesion and platelet aggregation: E-selectin, ICAM-1 Integrins, Glycoprotein IIb, Il-2, IL-4, IL-1beta, TGF-beta.

Group 5. This is a group of proteins involved in cell signaling and cell cycle regulation: PPAR-gamma, Cyclin D1 Cyclin E, Bcl2-L1, p27, CD95 (APO-1/Fas ligand).

The expression of P27, Bcl2, alpha-TTP, Cytochrome P450 (CYP3A), gamma-glutamyl-cysteine sythetase heavy subunit, tropomyosin, Il-2 and CTGF appears to be upregulated by one or more tocopherols. All the other listed genes are down-regulated. Several mechanisms may be implicated in tocopherol depend gene regulation. In some cases protein kinase C has been involved, due to its deactivation by alpha-tocopherol and its participation in the regulation of a number of transcription factors (NF-κB, AP1). In other cases a direct involvement of PXR/RXR has been documented. The ARE appears in some cases also implicated as well as the TGF-beta-RE. This heterogeneity of mediators of tocopherol actions suggests the need of a common denominator that could be a receptor or a co-receptor, able to interact with tocopherol on one end and with transcription factors on the other directed toward specific regions of promoter sequences of sensitive genes. The search for this mechanism and the details of the features involved in this central signaling mechanism are the object of current investigations in our as well as in other laboratories.

α-Tocopherol and endothelial nitric oxide synthesis

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Nitric oxide (NO) is a central regulator of vascular tone and homeostasis and is involved in the antiatherosclerotic properties of endothelial cells. The activation of endothelial NO synthase (eNOS) is mediated by an increase of intracellular calcium and/or by enzyme phosphorylation/dephosphorylation which is determined by each given stimulus. The present study in human umbilical vein endothelial cells investigates the role of α-tocopherol in the regulation of ionomycin-induced NO synthesis. The latter involves phosphorylation of the endothelial NO synthase at its serine residue 1177 which is mediated by calmodulin-dependent kinase-II (CaMKII) and to a minor extent by protein kinase A (PKA). Preincubation of cells with α tocopherol (10 – 200 μM, 24 h) increased ionomycin-induced eNOS phosphorylation at serine 1177 which corresponded to an increased citrulline and cGMP formation (both indicators for NO production) in intact cells. The potentiation of serine 1177 phosphorylation was dependent on the hydrophobic structure of α-tocopherol since it was mimicked by γ-tocopherol but not by trolox, a hydrophilic derivative of α-tocopherol lacking the phytyl side-chain. CAMKII and PKA inhibition reduced ionomycin-stimulated serine phosphorylation in α tocopherol-treated cells to a lower extent than in control cells suggesting that α tocopherol may affect additional protein kinases, phosphatases or eNOS interactions with regulatory proteins. The effects of α tocopherol on NO formation and eNOS phosphorylation were amplified by coincubation with ascorbate (100 μM, 24 h) which might regenerate oxidized α tocopherol and act synergistically with α tocopherol. Our data suggest that α tocopherol-mediated potentiation of eNOS activation may contribute to the prevention of endothelial dysfunction *in vivo*.

Identification of the molecular mechanism of actions of alpha-tocopherol in rat liver by global gene expression profile analysis

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The recent discovery that vitamin E (VE) regulates gene activity at the transcriptional level indicates that VE may exert part of its biological effects by mechanisms which are independent of its well-recognised antioxidant function. The objective of this study was the identification of hepatic vitamin E sensitive genes and examine the effects of VE on their corresponding biological endpoints. Two groups of male rats were randomly assigned to either a VE sufficient diet or to a control diet poor in VE for 290 days. High-density oligonucleotide microarrays comprising over 7,000 genes were used to assess the transcriptional response of the liver. Differential gene expression was monitored over a period of 9 months, at 4 different time-points, and rats were individually profiled. This experimental strategy identified several VE sensitive genes, which were chronically altered by dietary VE. Vitamin E supplementation down-regulated scavenger receptor CD36, coagulation factor IX and 5a-steroid reductase type1 mRNA levels while hepatic gamma glutamyl-cysteinyl synthetase was significantly up regulated. Measurement of the corresponding biological endpoints such as activated partial thromboplastin time, plasma dihydrotestosterone and hepatic glutathione substantiated the gene chip data which indicated that dietary VE plays an important role in a range of metabolic processes within the liver.

Antioxidants in Inflammation and Oxidative Stress Caused by Exercise

Maret G. Traber[1,2,3], Angela Mastaloudis[1,3], Jason D. Morrow[4], Dawn W. Hopkins[1,3], Sridevi Devaraj[5], Tian-Wei Yu[3], Robert P. O'Donnell[6], Roderick H. Dashwood[3] and Balz Frei[3]

Depts [1]Exercise & Sport Science, [2]Nutrition, [3]Linus Pauling Institute, [6]Dept Statistics, Oregon State U, Corvallis, OR; [4]Depts Medicine, Pharmacology, Vanderbilt U School of Medicine, Nashville, TN; [5]Dept Pathology, UC Medical Center, Sacramento, CA

To determine whether DNA damage, lipid peroxidation or inflammation are ameliorated by 6 w daily supplementation with 1000 mg vitamin C and 400 IU *RRR*-alpha-tocopheryl acetate, runners (n=22, 11 women, 11 men) were randomly assigned to placebos (PL) or antioxidants (AO) prior to running a 50 k ultramarathon. The % DNA damage (comet assay) increased at mid-race ($p<0.02$) and returned to baseline by 2 h post-race. One day post-race, AO women had 62% less DNA damage than PL women ($p<0.0008$); while AO had no effect in men. F_2-isoprostanes were 28 ± 2 (PL) and 27 ± 3 pg/ml (AO) at baseline, and increased during the run only in the PL group (41 ± 3 pg/ml, $p<0.001$). F_2-isoprostanes returned to baseline within 2 h in PL women, but remained elevated in PL men for 6 d ($p<0.03$). Running markedly increased inflammatory markers irrespective of AO status. Overall, endurance exercise resulted in DNA damage, lipid peroxidation, and increased inflammation. AO supplementation prevented endurance exercise-induced lipid peroxidation, had no effect on inflammation, but appeared to enhance DNA damage recovery in women but not in men.

Neurodegenerative Gene Networks in Mice Deficient in Tocopherol Transfer Protein and Vitamin E

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Functional importance of vitamin E (VE)in adults remains controversial. Its potent antioxidant properties suggest that VE is essential for the prevention of chronic diseases associated with oxidative stress. However, intervention studies with VE remain inconclusive. Importance of VE in humans is indicated by the discovery of patients with ataxia due to VE deficiency associated with mutations in tocopherol transfer protein (TTP) gene. Mice with deleted TTP gene are deficient in VE and show ataxia with ageing. We have used GeneChips to define genome-wide *in vivo* responses to chronic deficiency of VE in mice lacking the TTP gene. Differential analysis of mRNA profiles from livers and cerebral cortex of adult mice showed tissue specific responses to changes in TTP and VE. Bioinformatic analysis of differentially expressed genes indicated increased oxidant stress in livers and brains of VE/TTP-deficient mice. However, distinct gene-networks were affected in each tissue. The livers of deficient mice showed mRNA profile of cell proliferation and neo-vascularization whereas the cortex showed a neurodegenerative profile. Particularly noteworthy in the cortex was the repression of mRNAs encoding retinoic acid receptor related orphan receptor-alpha (ROR-α). ROR-α deficiency causes ataxia in mice and may account for ataxia in TTP deficient mice suggesting the importance of ROR-α in patients with ATTP mutations. Thus vitamin E likely modulates tissue specific gene-networks *in vivo* associated with behavioral deficits and it may exert some of its actions via member(s) of nuclear receptor superfamily.

Session III
Micronutrients: Flavonoids and Phytoestrogens

Molecular mechanism of green tea catechin inhibition of VEGF-induced angiogenesis.

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Green tea may prevent cancer development via several mechanisms. One of the mechanism is believed to be through its inhibition of tumor angiogenesis. Earlier, we have shown that the tea catechins including epigallocatechin gallate (EGCG) inhibits angiogenesis through inhibition of Akt and VE-cadherin phosphorylation and by suppression of production of proangiogenic cytokine, IL-8. To further elucidate the antiangiogenic mechanisms of EGCG, we investigated its regulation of other molecular processes in VEGF-induced angiogenesis *in vitro* including formation of an essential receptor complex, activity of PI3-kinase, induction of IL-8, and activation of NF-κB, a transcription factor necessary for IL-8 production. Immunoprecipitation and Western blot analysis revealed that EGCG (0.5-20 μM) dramatically inhibits the formation of the VEGFR2 receptor complex formed upon the binding of its ligand VEGF165. EGCG also significantly decreases the activity of PI3-kinase in a dose-dependent manner. Gel shift assay showed that EGCG also significantly decreased the activation and DNA-binding ability of NF-κB along with the suppression of IL-8 production at the mRNA (RT-PCR) and protein levels (ELISA). Our results suggest that EGCG inhibits angiogenesis by suppressing a variety of VEGF-induced angiogenic processes and presents novel mechanisms by which a dietary component can modulate pathological processes.

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Flavan-3-ols and Dimeric Procyanidins Inhibit NFκB Activation at Multiple Steps in Jurkat T Cells

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Flavonoids are natural substances present in plant foods that could provide health benefits. Such benefits are due in part to their antioxidant and anti-inflammatory properties. However, considering their submicromolar blood plasma concentrations, flavonoids could hardly compete in vivo with radical scanvengers as ascorbate or tocopherols. Chemically, flavan-3-ols constitute a sub-group of flavonoids, which major representatives are (-)-epicatechin and (+)-catechin, which are named procyanidins (PC). We investigated the capacity of the flavan-3-ols (-)-epicatechin (EC) and (+)-catechin (CT), and dimeric procyanidin (DP-B) to modulate phorbol 12-myristate 13-acetate (PMA)-induced NF-κB activation in Jurkat T cells. From the obtained results, we concluye that EC, CT, or DP-B can influence the immune response by modulating NF-κB activation. This modulation can occur at early steps (regulation of oxidant levels, IKK activation) as well as late steps (binding of NF-κB to DNA) of the NF-κB activation cascade. Supported with grants from University of Buenos Aires (B042), ANPCYT (PICT-01-08951), and CONICET (0738/98), Argentina. Mars Incorporated, Hackettstown, NJ, USA, provided the procyanidins for these studies.

Misfolding of apoB and Atherogenesis: Can Specific Isoflavones Protect by Stabilizing Protein Structure?

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Some early events of atherogenesis are attributed to "altered " structures of LDL that aggregate and interact with the extracellular matrix in the sub-endothelial space. The biological processing of these lipoproteins "poisons", as so-called "Trojan Horses", cells committed to their removal and activates an inflammatory response. An electronegative LDL (LDL-) represents a form of altered LDL, and may be a possible hallmark of disease. In LDL- apoB is misfolded. The apoB secondary structure exists in a dynamic state that is, in part, dependent on interaction with lipids. The alpha to beta shift in secondary structure in LDL- is attributed to an alteration of the water lipid boundary produced by different challenges, among which the most known is lipid peroxidation. This interpretation is in agreement with the finding that phospholipase A produces the same alteration in protein structure. LDL- is prone to aggregation in long filaments similar to those produced by typical amyloidogenic proteins, and, remarkably, antibodies to beta-amyloid recognize the misfolded LDL-. The notion of misfolding of apoB provides also some hints about the well-known protective effect of estradiol. The hormone binds specifically and with high affinity to a single binding site of apoB stabilizing its structure and shrinking the size of LDL as evidenced by CD and SAXS. A similar "protective" effect with respect misfolding aggregation and production of amyloidosis has been previouly observed with other proteins (e.g transthyretin) upon binding of specific ligands (thyroid hormones or retinol). This may fit a pattern for the stabilization of protein structure upon binding to specific ligands. Thus, a new role can be seen for molecules (drugs or nutrients) based on the stabilization of proteins, preventing misfolding. We have preliminary evidence that soy isoflavones, but not other polyphenols with strong antioxidant capacity (e.g. quercetin, catechin, epigallocatechin or procyanidins), reproduce the protective mechanism of estradiol by preventing the loss of alpha helix structure in LDL that can be produced by minimal modification processes.

Estrogens and Phytoestrogens Exert their Antioxidant Actions by Modulating Gene Expression –Importance in Aging

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Females live longer than males. This occurs in many species, including humans. Thus studying the mechanism of this difference may provide us with hints to understand the basic biology of ageing.

Mitochondria from females produce approximately half the amount of peroxides than those from males. Ovariectomy prevents this effect. Oestrogen replacement therapy restores it. Oestrogens do not act *in vivo* because of their *in vitro* antioxidant properties, but rather by up-regulating the expression of antioxidant, longevity-related genes such as that of glutathione peroxidase, superoxide dismutase, and cytochrome C oxidase. Some of these genes have oestrogen responsive elements in their promoter region. Others do not. To understand the mechanism of the beneficial effects of oestrogens, we studied the action in a mammary cell line in culture. Oestrogens activate the MAP kinase pathway. They increase phosphorilation of ERK 1 and 2 and of P38 MAP kinases. These, in turn, activate the NFKB signalling pathway which in turn activates genes such as that of superoxide dismutase and glutathione peroxidase.

Phytoestrogens are a family of compounds present in vegetable food which may have some of the beneficial effects of oestrogens without significant feminising activity. We have studied the effect of genisteine on the expression of longevity-related genes and found that it increases the expression of superoxide dismutase glutathione peroxidase amongst others. Thus, genisteine and other phytoestrogens may be useful to mimic the beneficial effects of oestrogens on longevity without some of their deleterious effects. The favourable roles of phytoestrogens on longevity and on age-related diseases require further investigation.

Session IV
Micronutrients

The Role of ROS and Antioxidants in Wound Healing during Diabetic Complications

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Although various factors are involved in the wound healing process, malnutrition may be a major factor that can lead to tissue damage and delay wound healing during diabetic complications. It is well known that diabetes and malnutrition cause compromised immune functions and decreased antioxidant defense in many studies. Thus, nutritional modulation of immune function and antioxidant status may play a crucial role in the control and regulation of wound healing during diabetic progression. ROS and proinflammatory cytokines produced by immune cells during inflammation activate NFκB that induces expression of chemokines and cytokines during the inflammatory stage. Notably, protein energy malnutrition (PEM) and Zn deficiency are well-known health problems associated with delayed wound healing. N-acetyl cysteine (NAC) supplementation in PEM may help wound healing by enhancing immune response and antioxidant defense. Zn supplementation may also increase immune function and antioxidant defense. The results of our experiments demonstrated that PEM impaired wound healing, possibly due to delayed neutrophil infiltration and decreased gene expression of cytokines. However, NAC or Zn supplementation restored neutrophil response and normalized gene expression of cytokines in the early inflammatory stage of cutaneous wound healing. Our study provides further evidence of the critical role of nutrition in wound healing during diabetic complications. More importantly, results from these experiments demonstrated that antioxidant supplementation provides an effective intervention strategy to enhance wound healing in diabetic patients

Selenium Delivery Proteins Can Provide a Relatively Non-Toxic Elemental Form of Selenium for Biosynthesis of Selenophosphate and Selenoenzymes

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Selenophosphate serves as selenium donor for synthesis of specific selenocysteine-containing enzymes in mammals and many bacteria. Selenophosphate synthetase from *Escherichia coli* can use high levels of free selenide as substrate for the ATP-dependent synthesis of selenophosphate *in vitro*. For certain isoenzyme forms that fail to use free selenide, an elemental, less toxic form of selenium serves as a substitute substrate. Elemental selenium is derived from free selenocysteine by the action of selenocysteine lyase or from a selenotrisulfide (RSSeSR) by rhodanese type enzymes. In general, the form of selenium donated by a lyase is a more effective substrate for selenophosphate synthetases than mM levels of free selenide. Three pyridoxal 5′-phosphate-dependent lyases from *Escherichia coli* that utilize both cysteine and selenocysteine as substrates forming elemental S and Se, respectively, have been characterized by other investigators. These lyase proteins exhibit sequence homology to the cysteine desulfurase (NifS) family of enzymes that provide sulfur for FeS cluster formation. However, specific selenium delivery systems that are able to effectively discriminate between mM levels of selenium and μM levels of sulfur compounds present in culture media clearly are essential for organisms that synthesize selenium-dependent enzymes needed for energy production and growth. One such microorganism, *Methanococcus vannielii*, synthesizes especially high levels of selenium-containing enzymes and seleno-tRNAs. *M. vannielii* proved to be a good source of a selenocysteine-specific lyase enzyme and a new selenium-binding protein. Some of the properties of these purified proteins will be described.

Flow Cytometric Analysis of the Oxidative Status of RBC and Platelets: The *In Vivo* Effects of Fermented Papaya Preparation (FPP) on β-Thalassemia Patients

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In sickle cell anemia and thalassemia (thal), although the basic lesion is in the globin genes, the pathology involves oxidative stress mediated cell damage. In addition, these patients occasionally develop thromboembolic phenomena, which is caused in part by increased platelet activation. To study the role of oxidative stress in these pathologies, we measured by flow cytometry Reactive Oxygen Species (ROS) and reduced glutathione (GSH) in RBC and platelets. β-thal RBC and platelets showed increased ROS and decreased GSH compared with normal cells, at basal state and following stimulation by oxidants (hydrogen peroxide and t-butyl-hydroperoxide). The oxidative status of these cells was increased by transferrin-bound and non-bound iron, hemin and phenyl hydrazine - a hemoglobin denaturation agent. These effects were inhibited by the antioxidants N-acetyl cysteine, vitamin C as well as an aqueous extract of Fermented Papaya Preparation (FPP). We then studied the effect of 6-week treatment of β-thal patients with FPP on the oxidative status of their RBC and platelets. Following treatment, ROS in both cells dropped, while GSH increased. Differences were statistically significant. These results suggest that FPP decreases oxidative stress of RBC and platelets *in vitro* and *in vivo*. The oxidative parameters were measured by flow cytometry - a standard technology in hematology labs. The *in vivo* effects observed suggest that FPP may have important clinical efficacy in thal as well as in normal and other pathological conditions.

The effects of vitamin C, flavonoids, and fructose on antioxidant protection of human plasma

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Dietary flavonoids and vitamin C may exert health benefits due to their antioxidant properties. To assess the effects of vitamin C on plasma antioxidant status and resistance to oxidative stress, plasma was obtained from 20 subjects before and 2 h after oral administration of 2 g of vitamin C. Plasma concentrations of ascorbate, but not other physiological antioxidants, increased significantly. Upon incubation of plasma with a free radical initiator (AAPH), ascorbate concentrations were positively correlated with the lag time preceding detectable lipid peroxidation. To evaluate the antioxidant effect of dietary flavonoids, aqueous apple extracts were added to human plasma followed by exposure to AAPH. 7.1 or 14.3 mg/ml total phenols of apple extract did not protect plasma ascorbate from oxidation, but significantly increased the half-life of urate and a-tocopherol and the lipid peroxidation lag time. Subsequently, six subjects consumed five Red Delicious apples or a fructose solution matching the fructose content of the apples. Plasma antioxidant capacity was measured before and up to 6 h after consumption as ferric reducing antioxidant potential (FRAP). Apple consumption caused an acute, transient increase in plasma FRAP, which was paralleled by a large increase in urate. Consumption of fructose mimicked the effects of apples on plasma FRAP and urate. Our data show that vitamin C supplementation and apple consumption increase the antioxidant protection of human plasma, which in the latter case is due to an increase in plasma urate, not apple-derived antioxidant flavonoids.

Alpha-Lipoic acid in health and disease: An overview

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To date alpha-lipoic acid is the only antioxidant supported by a complete set of preclinical and clinical data base for its efficacy and safety in the treatment of diabetic polyneuropathy. Alpha lipoic acid has shown in experimental diabetic neuropathy:

- improvement of endoneurial blood flow and other functional parameters of vascular dysfunction
- reduction of biochemical vascular dysfunction markers such as NFKB and von Willebrand factor
- reduction of imbalance between increased oxidative stress and reduced antioxidant levels
- improvement of endoneurial glucose utilization and phosphocreatine levels
- improvement of neurotrophic deficits

— all these effects leading to nerve function improvement. The pre-clinical effects have been verified in diabetic patients with neuropathy in order to rule out any molecular discrepancies between experimental and human diabetic polyneuropathy.

Seven placebo-controlled, double-blind, clinical studies have been performed showing effects of alpha-lipoic acid treatment on neuropathic symptoms, neuropathic deficits and nerve function parameters. A meta-analysis of four placebo-controlled studies has been conducted to investigate the overall efficacy in 1258 patients with diabetic polyneuropathy and showed that within 3 weeks alpha-lipoic acid can reduce neuropathic symptoms, while improving neuropathic deficits.

These data suggest that diabetic polyneuropathy is an oxidative stress linked disorder that can be successfully treated with alpha-lipoic acid.

New clinical studies comparing non diabetic and diabetic patients show that oxidative stress measures are especially elevated in diabetic patients with peripheral and autonomic neuropathy.

Recently, it has been found that a polymorphism in the SOD antioxidant gene may be associated with the development of diabetic polyneuropathy. Patients with the ARG/ARG or ARG/GLY genotype have a 2-3 fold higher risk to develop diabetic polyneuropathy compared to patients with a Gly/Gly phenotype who appear to be less prone to develop diabetic polyneuropathy.

Furthermore, patients with the ARG/ARG or ARG/GlY genotype have higher levels of oxidative stress measures than patients with the normal GLY/GLY genotype. These last results provide further evidence that diabetic poylneuropathy is an oxidative stress disorder, thus confirming the potential therapeutic effect of the antioxidant alpha-lipoic acid. Whether this genotype is pathologically involved in other disorders remains to be evaluated.

New preclinical findings indicate that alpha-lipoic acid may also be efficacious in other diabetic complications such as diabetic nephropathy and retinopathy. Preliminary studies suggest a nephroprotective effcet of alpha-lipoic acid after 18 month treatment

These findings confirm Michael Brownlees hypothesis that diabetes is an oxidative stress disorder and the antioxidant alpha-lipoic acid may represent an effective treatment option.

Up to now alpha-lipoic acid has not been clinically investigated in other

Redox Regulation of Heat Shock Protein Expression in Aging Brain: Protection by Acetylcarnitine

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Oxidative damage plays a crucial role in the brain aging process, and induction of heat shock protein (HSPs) is critically utilized by brain cells in the repair process following various pathogenic insults. We have recently focused our recent research on the role of the carnitine system in brain stress tolerance and antidegeneration. In the present study we investigated, in rats 6, 12 and 28 months old, and in rats 28 months old receiving acetylcarnitine (1g/kg/day) for 6 months, the role of heat shock signals on aging-induced changes in mitochondrial bioenergetics and antioxidant status. In all brain regions examined mRNA and protein synthesis of Hsp70 and Hsp60 increased with age up to 28 months; at this age the maximum induction was observed in the hippocampus and substantia nigra followed by cerebellum, cortex, and striatum. HSPs induction was associated with significant changes in glutathione (GSH) redox state, mt complex expression and HNE levels. Interestingly, treatment with LAC resulted in a marked decrease in HNE and DPNH content associated with increased protein expression and activity of Heme oxygenase-1, primarily in the hippocampus, cortex and cerebellum. Our results sustain a role for GSH redox state in Hsp expression. Remarkably, the increase of HO-1 expression could promote the functional recovery of oxidatively damaged proteins and protect cells from progressive age-related brain damage. Conceivably, therapeutic strategies focussing on LAC treatment, by up-regulating HO signal pathways and increasing bilirubin levels, may represent a crucial mechanism of defence against free radical-induced damage occurring in aging brain and in neurodegenerative disorders.

The 10 Basic Requirements for a Scientific Paper Reporting Antioxidant, Antimutagenic or Anticarcinogenic Potential of Test Substances in *In Vitro* Experiments and Animal Studies *In Vivo*

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There is increasing evidence that chemicals/test substances cannot only have adverse effects, but that there are many substances that can (also) have a beneficial effect on health. Food and Chemical Toxicology regularly publishes papers in this area. Since the journal has every intention in continuing to do so in the near future, it has become essential that studies reported reflect an adequate level of scientific scrutiny. Therefore a set of essential characteristics of studies has been defined. These basic requirements are default properties rather than non-negotiable: deviations are possible and useful, provided they can be justified on scientific grounds. The 10 basic requirements for a scientific paper reporting antioxidant, antimutagenic or anticarcinogenic potential of test substances in *in vitro* experiments and animal studies *in vivo* concern the following areas: (1) Hypothesis-driven study design, (2) The nature of the test substance, (3) Valid and invalid test systems, (4) The selection of dose levels and gender, (5) Reversal of the effects induced by oxidants, carcinogens and mutagens, (6) Route of administration, (7) Number and validity of test variables, (8) Repeatability and reproducibility, (9) Statistics, and (10) Quality Assurance. Food and Chemical Toxicology 41 (2003) 603-610

Session V
Coenzyme Q

Antioxidant activity of lipoprotein-associated ubiquinol 10

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Atherosclerosis, a major cause of cardiovascular disease, is characterized by heightened oxidative stress and oxidation of low-density lipoprotein (LDL) is implicated as a cause of atherosclerosis. As a result, there has been much interest in how LDL is oxidized and how this process is prevented by antioxidants. Studies on LDL antioxidation have focused on α tocopherol (TOH), the most active form of vitamin E and quantitatively the most abundant antioxidant associated with LDL lipid extracts. However, intervention studies using vitamin E supplements and aimed at reducing cardiovascular disease have yielded disappointing results, for reasons poorly understood at present. One possible reason is that TOH *alone* may not provide effective antioxidant protection for LDL. Indeed, *in vitro* studies have established that under some circumstances TOH fails to protect LDL against oxidation and, in fact, can promote LDL lipid oxidation. In this context, freshly isolated LDL also contains small amounts of ubiquinol 10 that represents the first line of LDL's antioxidant defence and that synergizes with and prevents the pro-oxidant activity of TOH. Dietary supplementation of humans with ubiquinone- 10 (the oxidized form of ubiquinol 10) increases LDL's content of ubiquinol 10 and the resistance of the lipoprotein to *ex vivo* oxidation. Similarly, in apolipoprotein E-deficient mice, dietary ubiquinone 10 increases ubiquinol 10 in circulating lipoproteins and the aorta, and significantly decreases the vascular concentration of lipoprotein-associated lipid hydroperoxides. Importantly, dietary supplementation with ubiquinone 10 also decreases the extent of atherosclerotic lesions in apolipoprotein E-deficient mice. The results show that supplemental ubiquinone 10 can inhibit lipoprotein lipid oxidation *in vivo*, and this may contribute to inhibition of atherosclerosis.

Ubiquinol/ubiquinone ratio as a marker of oxidative stress

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Reduced form of coenzyme Q (ubiquinol) has been suggested a front-li ne antioxidant against oxygen radicals [1-3]. When human plasma was incubate d under aerobic conditions, ubiquinol-10 depleted after the depletion of asc orbate [4]. However, no significant decay in alpha-tocopherol was seen in th is incubation [4]. A significant increase in cholesteryl ester hydroperoxide s was observed after the depletion of ascorbate and ubiquinol-10, indicating that alpha-tocopherol without ubiquinol-10 and ascorbate did not suppress th e formation of lipid hydroperoxide. Roland Stocker et al. showed that tocoph eroxyl radicals act as an chain carrier in this lipid oxidation and named th is oxidation as tocopherol-mediated peroxidation (TMP) [5]. Ascorbate and ub iquinol-10 are the most important, biological anti-TMP reagents [4,6]. The r edox status of plasma coenzyme Q-10 is a good indicator of oxidative stress since oxidative stress is defined as a disturbance in the prooxidant-antioxi dant balance in favor of the former. We therefore developed a simple and rel iable method for the simultaneous detection of plasma ubiquinol-10 and ubiqu inone-10 [7] and found an increase in oxidative stress in patients with hepa titis, cirrhosis, and hepatoma [8], inpatients treated with percutaneous tra nsluminal coronary reperfusion [9], in newborn babies [10], in the prostate of ACI/Seg rats (animal model of prostate cancer) [11], and in LEC rats (ani mal model of liver cancer) [12].

A. Mellors and A. Tappel, J. Biol. Chem., 241: 4353-4356 (1966).

2. R. Takayanagi, K. Takeshige, and S. Minakami, Biochem. J., 192: 853-860 (1980).

3. Y. Yamamoto, E. Komuro, and E. Niki, J. Nutr. Sci. Vitaminol., 36: 5 05-511 (1990).

4. Y. Yamamoto, M. Kawamura, K. Tatsuno, S, Yamashita, E. Niki, and C. Naito, “Oxidative Damage and Repair” Ed. K.J. A. Davies, Pergamon Press (199 1) pp 287-291.

5. V. W. Bowry and R. Stocker, J. Am. Chem. Soc., 115: 6029-6044 (1993).

6. P. K. Witting, C. Westerlund, and R. Stocker, J. Lipid Res., 37: 853 -867 (1996).

7. S. Yamashita and Y. Yamamoto, Anal. Biochem., 250: 66-73 (1997).

8. Y. Yamamoto, S. Yamashita, A. Fujisawa, S. Kokura, and T. Yoshikawa, Biochem. Biophys. Res. Commun., 247: 166-170 (1998). 9. Y. Yamamoto and S. Yamashita, BioFactors,

9. 241-246 (1999).

10. K. Hara, S. Yamashita, A. Fujisawa, T. Ogawa, and Y. Yamamoto, Bioch em. Biophys. Res. Commun., 257: 244-248 (1999).

11. Y. Homma , Y. Kondo, M. Kaneko, T. Kitamura, W. T. Nyou, M. Yanagisa wa, Y. Yamamoto, and T. Kakizoe, Carcinogenesis, in press.

12. Y. Yamamoto, H. Sone, S. Yamashita, Y. Nagata, H. Niikawa, K. Hara, and M. Nagao, J. Trace Elem. Exp. Med., 10: 129-134 (1997).

Coenzyme Q_{10} in Neurodegenerative Diseases

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Coenzyme Q_{10} (ubiquinone), which serves as the electron acceptor for complexes I and II of the mitochondrial electron transport chain and also acts as an antioxidant, has the potential to be a beneficial agent in neurodegenerative diseases in which there is impaired mitochondrial function and/or excessive oxidative damage. Substantial data have accumulated to implicate these processes in the pathogenesis in certain neurodegenerative disorders, including Parkinson's disease, Huntington's disease and Friedreich's ataxia. Although no study to date has equivocally demonstrated that coenzyme Q_{10} can slow the progression of a neurodegenerative disease, recent clinical trials in these three disorders suggest that supplemental coenzyme Q_{10} can slow the function decline in these disorders, particularly Parkinson's disease.

Effect of CoQ_{10} administration on myocardial function and vascular response in ischemic heart disease

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Myocardial levels of CoQ_{10} were found to be decreased in heart biopsies from patients in chronic heart failure (CHF) (1) and this constituted the rationale for using CoQ_{10} as coadjuvant therapy in cardiology. Clinical results are not unanimous, but there is growing evidence that CoQ_{10} administration positively affects heart function (2). In recent years the antioxidant properties of CoQ_{10} have been deeply investigated and it has also been pointed out that CoQ_{10}, in diabetic patients, is capable of improving endothelium-dependent dilation of the brachial artery (EDDBA), an NO-dependent vascular response (3). In ischemic heart disease patients this response is depressed and can be restored by exercise training (ET) (4).

In the present study we investigated 23 patients in NYHA class II and III (20M, 3W, mean age 59±9 years) with stable CHF due to ischemic heart disease (ejection fraction 37±7%), using a double-blind, placebo-controlled cross-over design. Patients were randomly assigned to each of the following treatments: oral CoQ_{10} (100 mg tid), placebo, CoQ_{10} plus supervised ET (60% of peak VO_2, 5 times a week), placebo plus ET. Each phase lasted 4 weeks. On study entry and at the end of each phase, all patients underwent a symptom-limited cardiopulmonary exercise testing (CPET), endothelium-dependent (ED) and endothelium-independent (EID) reactivity of the BA, low-dose dobutamine stress echocardiography, and blood chemistry. Both peak VO_2 and EDDBA improved after CoQ_{10} as compared with placebo (peak VO_2, baseline:17.4±3.6 ml/kg/min; CoQ_{10}: 19.6±3.6; placebo:17.9±3.8, $P<0.05$; EDDBA, baseline: 3.99±1.5%; CoQ_{10}: 5.6±1.9%; placebo: 4.2±1.96%, $P<0.005$). CoQ_{10} combined with ET determined greater improvements in peak VO_2 (+12%, $P<0.001$ vs CoQ_{10}), EDDBA (+47%, $P<0.001$ vs CoQ_{10}) and systolic wall thickening score index both at rest and peak dobutamine than CoQ_{10} alone. EID was unchanged after all treatments. CoQ_{10} supplementation resulted in a threefold increase in plasma CoQ_{10} level (from 0.81±0.13 μg/ml to 3.25±1.5 μg/ml, $P<0.0001$), while the combination with ET increased plasma Q_{10} level by four times (4.1±2.1,$P<0.0001$ vs placebo), suggesting a role for ET to increase plasma CoQ_{10} availability. No side effects were reported with CoQ_{10}. No significant changes were observed in LDL, HDL, total cholesterol, triglycerides, uric acid and vitamin E after CoQ_{10} alone or in combination with ET. In conclusion, oral CoQ_{10} improves peak VO_2, the EDDBA and LV contractility of CHF patients without any side effects. The combination of CoQ_{10} and ET resulted

in higher plasma CoQ_{10} levels and more marked improvements than CoQ_{10} or exercise given alone. Along with its well known role in mitochondrial bioenergetics, CoQ_{10} also improves endothelial function in CHF, possibly by strengthening NO effect.

References

1. Mortensen S.A., Vadhanavikit S., Baandrup U., Folkers K. Long-term coenzyme Q_{10} therapy: a major advance in the management of resistant myocardial failure, Drugs Under Exp & Clin. Res, 1985;11: 581-584.
2. Langsjoen P and Langsjoen A. Overview of the use of CoQ_{10} in cardiovascular disease. Biofactors, 1999; 9: 273-284,
3. Watts GE, Playford DA, Croft KD, Ward NC, Mori TA, Burke V. Coenzyme Q_{10} improves endothelial dysfunction of the brachial artery in Type II diabetes mellitus. Diabetologia, 2002;45: 420-426.
4. Belardinelli R, Georgiou D, Cianci G, Purcaro A. Randomized, controlled trial of long-term moderate exercise training in chronic heart failure: effects on functional capacity, quality of life, and clinical outcome. Circulation 1999; 99:1173-82.

Session VI
Redox Signaling and Gene Expression

Redox regulation of transcription factor activity

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Key regulators of the bacterial and yeast defenses against oxidative stress, respectively, are the OxyR and Yap1 transcription factors. Oxidation of the OxyR protein results in the formation of one intramolecular disulfide bond and leads to a change in OxyR DNA recognition. Structural studies of the OxyR redox domain showed that formation of the C199-C208 disulfide bond is associated with structural remodeling that reorients the DNA binding domain. In contrast, oxidation of the Yap1 protein results in the formation of two intramolecular disulfide bonds and leads to a change in Yap1 subcellular localization. In the absence of oxidative stress, Yap1 is localized to both the cytoplasm and the nucleus. Upon oxidative stress, Yap1 accumulates in the nucleus. We have recently solved the NMR structure of the Yap1 redox domain. These studies revealed that the C303-C598 and C310-C629 disulfide bonds along with interactions between conserved hydrophobic amino acids function to lock the nuclear export signal of Yap1 into the hydrophobic core of the protein. Upon reduction, the redox domain adopts an unfolded random coil conformation allowing for exposure of the nuclear export signal. The similarities and differences in the redox regulation of OxyR and Yap1 activity as well as the implications for other redox-sensitive proteins will be discussed.

Regulation of NAD metabolism: A model for the pyridine nucleotide cycle (PNC)

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Pyridine nucleotides play varied and occasionally conflicting roles in bacterial metabolism. While NAD serves primarily to carry electrons from oxidative (degrading) reactions to the electron transport chain, it also activate s DNA ends for ligation during replication and repair. In contrast to these essential roles, NADH is toxic during oxidative stress due to its role in production of hydroxyl radicals. NADPH serves primarily to carry electrons to reductive (biosynthetic) reactions, but resists oxidative stress by its contribution to reduction of glutathione and synthesis of deoxynucleotides. Two observations suggest regulatory changes in pyridine nucleotide metabolism following oxidative stress and DNA damage. Swenson observed that UV irradiation leads to a respiration block and loss of NAD pools. Olivera and co-workers documented an oxygen-stimulated cyclic pathway (PNC) whereby NAD (or NADP) is split to NMN and recycled to NAD. Pyridine metabolism is controlled at least four points. The first biosynthetic enzyme is feedback inhibited by NAD and the second is oxygen-sensitive. The trifunctional NadR protein responds to NAD by repressing two biosynthetic genes; in the absence of NAD these genes are expressed and the NadR protein gains two enzymatic activities, that contribute to assimilation of NmR. Finally the NADP synthetic enzyme, NAD kinase, is feedback-inhibited by NADPH. Under normal growth conditions, this kinase is nearly completely inhibited. We propose a model whereby this feedback control serves to increase NADPH levels during either oxidative stress or following DNA damage; this activity may underlie the oxygen-stimulated PNC.

Inhibition of apoptosis in acute promyelocytic leukemia cells leads to increases in the levels of oxidized protein and catalytically inactive 20-S proteasome

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The importance of apoptosis in organ function is highlighted by the facts: (a) that reactive oxygen-mediated oxidation of cellular enzymes/proteins often leads to loss of biological functionality and (b) that, after reaching maturity animal organs cease to increase in size, due to inhibition of cell replication activities. It follows that maintenance of optimal organ function is dependent upon the elimination of oxidatively damaged cells and their replacement with new cells. The present study was undertaken to test the proposition that the use of hydrogen peroxide to activate cell signaling pathways provides a mechanism for sensing abnormal increases in the levels of the reactive oxygen species and sets in place the ability to eliminate oxidatively damaged cells by apoptosis and to replace the bad cells with good cells by simultaneous activation of the cell replication capacity. If valid, this proposition raises the possibility that the accumulation of oxidatively modified proteins that occurs during aging and in various diseases might reflect decreases in the capacity to get rid of oxidatively damaged cells by apoptosis. To examine the effects of oxidative stress and apoptosis on the accumulation of oxidized protein, we exposed acute promyelocytic leukemia cells to hydrogen peroxide plus iron in the presence and absence of a general caspase inhibitor (Z-VAD-fink), which is known to inhibit caspase-induced apoptosis. Results of these studies show that inhibition of apoptosis leads to the accumulation of oxidatively modified proteins (protein carbonyls) and also to a substantial increase in the level of catalytically inactive 20-S proteasome protein.

The Balance between Cu, Zn SOD and Nitric Oxide in Amyotrophic Lateral Sclerosis

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Recent in vitro studies have suggested that the oxidizing and nitrating potential of peroxynitrite is only important when the fluxes of superoxide and nitric oxide are nearly equal. Biologically, the competition between SOD and nitric oxide is far more important determinant. Rate constants predict that NO is about 6 fold more efficient at competing with Cu,Zn SOD for superoxide. With activated macrophages, exogenous SOD does not stop peroxynitrite formation and in fact can catalyze tyrosine nitration. In a motor neuron, SOD is about 10 μM and as high as 140 μM with transgenic overexpression in mice. In contrast, NO levels are generally under 0.1 μM. Even with a 1,000 fold excess of SOD, one finds substantial amounts of nitric oxide can still converted to peroxynitrite. Kinetic simulations indicate that reoxidation of high concentrations of SOD by oxygen can make a substantial contribution towards the formation of peroxynitrite. When SOD loses zinc, the copper becomes far more reactive with endogenous antioxidants including ascorbate. Reoxidation of zinc-deficient SOD can become a major source of peroxynitrite when endogenous generation of superoxide is relatively low in the cytosol (less than 1% of total cellular oxygen consumption). These observations offer a reasonable biochemical mechanism for how wild-type SOD can participate in ALS and why mutations to SOD are dominant in familial ALS.

Proatherogenic Gene Modulation by Cholesterol Oxidation Products In Vascular Cells

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Purpose: Hypercholesterolemia is certainly associated to progression of atherosclerosis, but the mechanisms by which cholesterol contributes to the disease process are still quite undefined. Oxysterols, oxidation products of cholesterol, are by far more reactive than the parent compound. We deemed important to investigate their potential pro-inflammatory role in atherosclerosis. *In vitro* model used: cells of the macrophage lineage were challenged with the oxysterols more represented in human LDL and atherosclerotic plaques, at concentrations of pathophysiological interest.

Results: a mixture of oxysterols compatible with those found in LDL is able to up-regulate the expression of a number of genes related with monocyte chemoattraction, differentiation and adhesion, by this way sustaining a chronic inflammatory process within the atherosclerotic plaque. Notably, such a biologically representative mixture of oxysterols does not show the typical pro-apoptotic action exerted by defined oxysterols when singularly added to cultivated cells. Further, neither pro-apoptotic nor pro-inflammatory effects were observed when macrophage cell lines were challenged with unoxidized cholesterol.

Conclusions: 1) oxysterols rather than the parental compounds appear able to sustain an inflammatory process; 2) oxysterols present in mixture as in LDL of hypercholesterolemic patients, may act as a Trojan horse, not inducing direct toxic effect on vascular cells, but on the contrary building up on the sly pro-inflammatory stimuli.

The Mycobacterial Antioxidant Defense

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Mycobacterium tuberculosis is equipped with catalase and peroxiredoxins to cope with the host's oxidative challenge during the infection process. Disruption of the *katG* gene resulted in avirulence, while KatG-deficient clinical isolates were reported to be virulent due to constitutive overexpression of the peroxiredoxin AhpC. These observations revealed the relevance of both, catalase- and peroxiredoxin-dependent peroxide detoxification in mycobacteria. Moreover, *katG*$^-$-strains are known to be INH resistant, because catalase is required to activate INH to a tuberculocidal agent. Yet despite the obvious clinical impact of mycobacterial peroxiredoxins their metabolic context had remained obscure. By heterologous expression of mycobacterial genes encoding disulfide reductases, thioredoxin- and glutaredoxin-related proteins and different peroxiredoxins we created a tool box to reconstitute efficient mycobacterial peroxidase systems *in vitro*. The most appealing ones emerging from this puzzle were: i) thioredoxin reductase (*Mt*TR), thioredoxin C (*Mt*TrxC) and *Mt*AhpC; ii) *Mt*TR, *Mt*TrxB and the atypical two-cys peroxiredoxin *Mt*Tpx and iii) *Mt*TR, *Mt*TrxC and *Mt*Tpx. Surprising was the specificity of *Mt*TR for *Mt*TrxC and B (not TrxA or any of the other proteins with CXXC motifs) and of the Trxs for the peroxiredoxins. Another surprise was the peroxidatic efficiency of *Mt*TPx that, in terms of H_2O_2 and peroxynitrite reduction, was substantially higher that of *Mt*AhpC. Further considering proteome-based estimates of in vivo concentrations of components, the *Mt*TR/*Mt*TrxC/*Mt*TPx systems has to be rated as the most relevant one.

Oxidative Stress, Lysosomal Iron, and DNA Damage

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Oxidative DNA damage is known to involve site-specific Fenton-type chemistry, although presence of transition metals in the nucleus has not been convincingly shown. Recently, it was proposed that a major part of the cellular pool of loose iron is confined within the acidic vacuolar compartment. Consequently, rupture of secondary lysosomes, followed by relocation of labile iron to the nucleus, could be an important intermediary step in the generation of oxidative DNA damage. We employed the potent iron chelator desferrioxamine (DFO) conjugated to starch to form a high molecular weight DFO-complex (HMW-DFO) that will enter cells only by fluid phase endocytosis and remain within the acidic vacuolar compartment, thus chelating redox-active iron exclusively inside the endosomal/lysosomal compartment. HMW-DFO protected lysosomal membrane integrity against H_2O_2-induced oxidative disruption and prevented hydrogen peroxide-induced strand breaks in nuclear DNA. To exclude that lysosomal hydrolases, rather than iron, caused the observed DNA damage, limited lysosomal rupture was induced using the lysosomotropic detergent O-methyl-serine dodecylamine hydrochloride (MSDH). The findings suggest that oxidative damage to cellular DNA is minimal in the absence of redox-active iron and is mainly derived from intra-lysosomal iron translocated to the nucleus following lysosomal rupture.

Mitochondrial Glutaredoxin Knockdown and Apoptosis

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Glutaredoxins belong to the thioredoxin fold superfamily and catalyze glutathione-dependent oxidoreductions. The recently discovered mitochondrial and nuclear glutaredoxin (Grx2) differs from the more abundant cytosolic glutaredoxin (Grx1) by its higher affinity towards S-glutathionylated proteins and by being a substrate for thioredoxin reductase. Here, we have successfully established a method to silence the expression of Grx 2 in HeLa cells using short interfering RNA to study its role in vivo. Cells with levels of Grx2 below 3 % of the control were dramatically sensitized to cell death induced by doxorubicin/adriamycin and phenylarsine oxide, but they did not show signs of a general increase in oxidative damage. The ED50 for doxorubicin dropped from 40 µM to 0.7 µM and for phenylarsine oxide from 200 nM to 5 nM. No differences where seen when the cells where treated with cadmium, a known inhibitor of Grx1. These results indicate a crucial role of hGrx2 in the regulation of the mitochondrial redox status and in regulation of cell death at the mitochondrial checkpoint.

Oxidative stress gene modulation in mesothelioma as assessed by microarray *in-vitro, ex-vivo,* and *in-situ* analysis

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Asbestos fibers are known as mutagenic and carcinogenic for human and are responsible for occupational pulmonary diseases including asbestosis, bronchogenic carcinoma and malignant pleural mesothelioma, the cancer of the external lining of the lung. Transcriptomes of pleural cells and malignant pleura human samples (mesotheliomas) were compared by the use of microarrays. Gene expression profiling was obtained from i) cultured pleural cells (7,000 genes, in-vitro study), ii) tumor specimens (10,000 genes, ex-vivo study), and iii) microdissected pleural cells (10,000 genes, in-situ study). Results showed dozen of overexpressed genes in mesothelioma that (i) promote local invasion, (ii) protect cells against oxidative stress and (iii) counteract anti-cancer therapies. In the 3 studies, both FTL (ferritin light chain: HUGO symbols) and TXN (thioredoxin) overexpressions were consistently associated with the acquisition of the malignant phenotype. In microdissected cells, the expression of other redox-modulated genes, including FTH1 (ferritin heavy chain), GSTP1, MGST3 (microsomal glutathione S-transferase3), PRDX1 (peroxiredoxin1), PRDX4, SOD1, TXNL2 was increased in mesothelioma cells conferring them some resistance against ROS induced by chemotherapy. The portrait of normal and cancerous pleura achieved at the mRNA level seems meaningful for the understanding of asbestos-mediated carcinogenesis, as for as mesothelioma stratification and management. Indeed the mesothelioma markers described in this study should improve the accuracy of mesothelioma diagnosis and therapy.

Heme oxygenase-1 as a novel therapeutic target of drugs and micronutrients in the endothelium

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Heme oxygenase-1 (HO-1) has recently been recognized as protein with antioxidant and cytoprotective functions. We have shown that among different nitric oxide (NO) donors the organic nitrate pentaerythritol tetranitrate (PETN) is the most potent inducer of HO-1 expression in endothelial cells (Oberle et al., 2002, BBRC 290: 1539-44). Stimulation of HO-1 expression and activity (bilirubin or carbon monoxide formation) by NO donors, cGMP analogs or atrial natriuretic peptide was associated with increased resistance of endothelial as well as renal cells to oxidant injury (Polte et al., 2002, Free Radic. Biol. Med. 32: 56-631). HO-1 was found to be sensitive to induction not only by NO but also through aspirin. We observed activation of HO-1 protein synthesis in endothelial cells at low, antithrombotic concentrations of aspirin, presumably via NO-dependent pathways (Grosser and Schröder, 2003, Arterioscler. Thromb. Vasc. Biol. 23:1345-1351). Moreover, micronutrients such as vitamin C and the amino acids L-alanine, L-serine and L-methionine increased expression of HO-1 or/and ferritin, a secondary antioxidant protein that is often induced in tandem with HO-1. Increases in gene expression caused by the latter agents were also followed by sustained antioxidant protection of endothelial cells. Our results suggest induction of the HO-1/ferritin system as novel mechanism by which drugs as well as micronutrients may prevent cellular injury in atherosclerosis and other inflammatory processes.

Redox Regulation of T Cell Activation by Thioredoxin and TBP-2/VDUP-1

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Human thioredoxin (TRX) was originally identified as Adult T-cell leukemia derived factor (ADF) from human T-cell leukemia virus positive T-cell lines. TRX is induced by a variety of oxidative stress, and has been shown to play crucial roles in the regulation of cellular responses. A unique property of TRX is its releasing mechanism depending on redox-active site through a leaderless pathway. TRX may play important roles in tuning redox status of microenvironment[2]. We generated TRX-C35S to clarify the TRX membrane traffic mechanism. Unexpectedly, TRX-C35S entered rapidly into HTLV-I-transformed T Cells via the Lipid Raft System. Moreover, this entry of TRX-C35S might be restricted in the cells expressing IL-2R-alpha. Jurkat T cells activated by PMA and Ionomycin were increased to internalize this molecule. These results suggest that extracellular TRX can enter into intracellular compartment via Lipid Raft pathway to regulate cellular redox status. We identified thioredoxin-binding protein-2-vitamin D3 up-regulated protein 1 (TBP-2) as a negative regulator of TRX. Expression of TBP-2 was completely abolished in HTLV-I-positive IL-2-independent cells, whereas that of TRX was markedly enhanced. Further, ectopic overexpression of TBP-2 in the cells resulted in growth suppression accompanied by a decrease of TRX activity. These results suggest that TBP-2 plays a crucial role in the growth regulation of T-cells through the interaction with its target molecules including TRX[3].

[1] Annu. Rev. Immunol. 15, 351-, 1997

[2] J. Immunol. 172, 442-, 2004

Session VII
Carotenoids and Retinoids

Chemical and Metabolic Oxidation of Carotenoids

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To date, a wide range of carotenoids have been isolated, identified, and quantified from the extracts of fruits and vegetables commonly consumed in the U.S. These studies have revealed that 40 to 50 carotenoids may be available from the diet and absorbed, metabolized, or utilized by the human body. However among these only 13 all-E (trans)- and 12 Z (cis)-carotenoids are routinely found in human serum, milk, and tissues. In addition, there are 8 carotenoid metabolites resulting from three dietary carotenoids, namely, (3R,3'R,6'R)-lutein, (3R,3'R)-zeaxanthin, and lycopene. These metabolites appear to result from a series of oxidation-reduction reactions that involve modification of the end-groups of lutein, zeaxanthin, and lycopne while the polyene chain of these carotenoids remain intact. However, the nature of the products obtained by chemical oxidation of carotenoids is dependent on the oxidizing reagent and reaction conditions. Under controlled conditions, chemical oxidation can lead to modification of carotenoid end-groups and/or cleavage of the polyene chain to form a number of apocarotenals. Meantime, the observed biological activity of dietary carotenoids in certain cell culture studies have been attributed to the production of apocarotenals that are presumably formed from their parent carotenoids as a consequence of air oxidation. To clarify this point, the stability of a sample of purified lutein exposed to air at room temperature for two weeks has been investigated and the oxidation products of this carotenoid have been tentatively identified by HPLC-UV/Vis-MS.

Raman detection of carotenoids in human tissue

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Carotenoid compounds are important ingredients for the anti-oxidant defense system of the human body. These lipophilic molecules are potent free radical quenchers accumulated in the body tissues through fruit and vegetable consumption. Numerous epidemiological and experimental studies have shown that a higher dietary intake of carotenoids may protect against cancer, age-related macular degeneration, pre-mature skin aging and other pathologies associated with oxidative cell damage. The rapid and non-invasive measurement of carotenoid concentrations in human tissue therefore may be of diagnostic help.

We have used a noninvasive optical technique, based on resonance Raman spectroscopy, to rapidly screen carotenoid levels in human skin and in the retina and in this way to assess antioxidant status in large populations. Data obtained for a population of 1,375 subjects with a portable skin Raman scanner revealed that carotenoids are a good indicator of antioxidant status or oxidative stress. The study showed that people with high oxidative stress (for example smokers and people with high sunlight exposure) generally have low skin carotenoid levels, independent of their dietary carotenoid consumption. Using an ocular Raman instrument suitable for clinical studies we measured lutein and zeaxanthin concentrations in the living human retina of healthy and macular degeneration subjects. We find the Raman technique to be precise, specific, sensitive, and well suitable for clinical as well as field studies. It may become a useful method for the correlation between tissue carotenoid levels and risk for malignancies or other degenerative diseases associated with oxidative stress.

Resistance to Oxidative Stress Following Carotenoid Supplementation in Humans

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Oxidative stress may be implicated in the etiology of chronic diseases of aging such as cancer and cardiovascular disease. Dietary supplementation with antioxidants can be a part of a defense strategy to minimize oxidative damage in vulnerable population such as the elderly. Resistance to oxidative stress in healthy women (n=37, 50-70 yr) in response to an 8-week period of carotenoid supplementation was determined. Subjects were randomly assigned to one of 5 groups to take daily dose of either a combination of carotenoids (4 mg each of lutein, β-carotene and lycopene) or a single carotenoid (i.e., 12 mg each of lutein, β-carotene, or lycopene) for 8 weeks in a double-blind randomized trial. On study days 1, 15, 29, 43 and 57, overnight fasting bloods were collected. Lymphocytes were separated immediately after blood collection and analyzed for oxidative DNA damage. A single cell gel electrophoresis (comet) assay was used to determine endogenous DNA damage, as well as hydrogen peroxide induced DNA damage. All carotenoid supplemented groups showed significantly lower endogenous DNA damage at day 57 as compared to that of baseline ($p<0.005$), while the placebo group did not show any change. In particular, the endogenous DNA damage in the mixed carotenoid supplemented group was significantly reduced after only 15 days ($p=0.002$). When DNA was challenged with hydrogen peroxide, DNA damage was significantly decreased at day 57 as compared to that of day 1 in the mixed carotenoid group, β-carotene group and lycopene group, while no change was observed in the placebo or lutein groups. Our results indicate that carotenoid supplementation can effectively protect against DNA damage and that the protective effect of mixed carotenoid supplementation against DNA damage is rapid and consistent.

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Formation and Biological Effects of Beta-carotene Cleavage Products in view of the Particular Conditions in Inflamed Lung Tissue

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beta-Carotene (BC) has been used as supplement in food and medicine. However, intake of BC failed in clinical efficacy trials and even harmful effects were seen. Recent work has shown that the negative effects seen in smokers might be related to degradation of carotenoids to oxidative cleavage products (CP) having a high reactivity towards biomolecules. In previous studies we could show that mixtures of BC CP led to rapid loss of Na-K-ATPase activity and to impairment of ADP-stimulated respiration in rat liver mitochondria. Although a number of these BC CP were already identified the mechanism of their formation *in vivo* is still unknown. One possibility of oxidative non-enzymatic cleavage of BC in living organisms is HOCl/-OCl produced by polymorphonuclear leukocytes (PML). In *in vitro* experiments stimulated PML in culture were able to cleave BC present in the media in physiological concentrations. After degradation by PML a number of long-chain apo-carotenals but also of short chain BC CP could be identified. In further experiments we could show that these CP were in turn also able to influence PML. While BC CP in low concentrations (0.1 – 5 µM) were able to activate the superoxide radical production via NADPH-oxidase of PML, higher concentrations (≥ 20 µM) led to inhibition of the enzyme. These are interesting findings especially in view of the discussions of the unexpected pro-oxidative effects of BC in the intervention trials. It might be possible that in inflamed lung tissue which contains an increased number of phagocytosing cells BC will be degraded to CP when present in increased tissue concentrations. The knowledge about this is essential for the establishment of conditions for save carotenoid supplementation in disease prevention and clinical therapy.

Lycopene and Prostate Health

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Lycopene may inhibit growth of prostate tumors, however, its effects on the benign prostate are unclear. We studied in vitro whether lycopene inhibits growth of normal human prostate epithelial cells. Synthetic lycopene (5 μmol/L) inibited cell proliferation and cell cycle progression. Analysis of gene expression using Affimetrix gene arrays did not result in significant changes in cell cycle related genes. In contrast, protein levels of cyclin D1 were significantly inhibited by lycopene. These results suggest that lycopene may modulate growth of the benign prostate. In a second study it was investigated whether synthetic lycopene alone or in combination with vitamin E can inhibit prostate tumor growth in an animal model of prostate cancer. The animals were fed with synthetic lycopene, synthetic vitamin E, a combination of lycopene plus vitamin E, or placebo. Tumor growth was monitored via transrectal ultrasonography. Lycopene at a dose of 5 mg/kg b.w. significantly inhibited tumor growth and increased survival compared to the placebo group. This effect was increased when lycopene plus vitamin E (each 5 mg/kg b.w.) was fed to the animals. Lycopene and vitamin E may act synergistically in the inhibition of prostate cancer.

Session VIII
Carotenoids and Retinoids

Carotenoids and Gene Regulation by the Anti-Oxidant Response Element

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The anticancer activity of carotenoids involves interference in several pathways related to cancer cell transformation and proliferation and includes changes in the expression of proteins participating in these processes. We suggest that carotenoids and their oxidized derivatives interact with a network of transcription systems at low affinity and specificity. Induction of phase II detoxification enzymes is an important mechanism by which phytonutrients prevent cancer. Expression of phase II enzymes, such as NAD(P)H:quinone oxidoreductase (NQO1) and γ-glutamylcysteine synthetase (GCS), is regulated by the antioxidant response element (ARE), which is found in the promoters of genes encoding these proteins. The transcription factor Nrf2 binds to the ARE and induces the expression of phase II enzymes. We found that in transiently transfected cancer cells lycopene transactivated the expression of a reporter gene fused with ARE sequences. An increase in NQO1 and GCS protein and mRNA levels was observed in non-transfected cells after carotenoid treatment. The increase in these proteins was abolished by a dominant negative Nrf2, suggesting that carotenoid induction of phase II enzymes depends on a functional Nrf2 and the ARE transcription system. Moreover, Nrf2, which is found predominantly in the cytoplasm of control cells, translocated to the nucleus after treatment with some carotenoids (immunohistochemistry). Our results suggest that the ARE-driven induction of phase II enzymes represents a novel molecular mechanism for the cancer-preventive action of a diet rich in carotenoids.

Cleave it or leave it: Carotenoid metabolism and its impact on vision, cell differentiation, and development

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Vitamin A and its derivatives (retinoids) are essential components in vision, they also contribute to pattern formation during development and exert multiple effects on cell differentiation with important clinical implications. Animals, in general, cannot synthesize vitamin A de novo, but rely on a supply of dietary precursors. All naturally occurring vitamin A derives by enzymatic oxidative cleavage from carotenoids with provitamin A activity. This process follows a universal scheme in animals. To become biologically active, carotenoids must first be absorbed, then delivered to the site of action in the body, and in the case of the provitamin A function, metabolically converted. In the fruit fly Drosophila – with vitamin A functions being restricted to the visual system – blind mutants with impairments in this pathway have been isolated. The eye phenotype of ninaD flies is characterized by an absence of both carotenoids and retinoids, while in ninaB flies only retinoids are missing and carotenoids are highly accumulated. Our analyses revealed that the ninaD gene encodes a cell surface receptor rendering carotenoids available cellularly. NinaB, a beta,beta-carotene-15,15'-oxygenase, catalyzes the oxidative cleavage of the provitamin A, the key step in vitamin A formation, to give two molecules of vitamin A aldehyde (retinal). By searching for similar genome sequences in vertebrates, we identified three ninaB homologous genes. By loss-of-function experiments in the zebrafish, we addressed their roles in embryonic vitamin A metabolism. These analyses revealed that we identified a novel family of carotenoid/retinoid metabolizing enzymes with specific functions in retinoic acid signalling as well as in the retinoid (visual) cycle of the eyes.

Macular Carotenoids in Eye Health

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The macular pigment (MP), consisting of lutein (L) and zeaxanthin (Z), is a feature of human and other primate retinas. The carotenoids are derived from the diet and transported from the blood serum to the retina where some of the L appears to be converted to meso-Z. The MP layer screens blue light and may therefore protect underlying structures from damage resulting from reactive oxygen species (ROS) generated by photosensitized reactions. In addition, it may quench ROS. Oxidative processes are implicated in the etiology of age-related macular degeneration (AMD). Thus it is not surprising that higher levels of L and Z in the diet, serum and retina are associated with a decreased risk of AMD. We are conducting a supplementation study to determine the MP response to doses of 0 (placebo), 5, 10 and 20 mg of L/day for 120 days in age-matched subjects, 18 to 30 years old. MP optical density (OD) is measured by heterochromatic flicker photometry (HFP). The average rates of increase in MPOD that we observe are -0.090 ± 0.432, -0.078 ± 0.596, 0.383 ± 0.720 and 0.490 ± 0.630 mAU/day for the 0, 5, 10 and 20 mg groups respectively. For a separate group of older subjects (>50 years) taking 20 mg/day, the rate is lower (0.132 ± 0.656 mAU/day) than for the younger group taking this dose. Because HFP is difficult for some subjects, particularly those with compromised vision, we are developing an objective method for determining MPOD that is based on a modified retinal camera. An advantage of the photographic method is that it provides the distribution of MPOD in the retina rather than an average value in, say, the central 1.5°, as provided by our flicker photometer.

Lutein Effects on Nervous Tissue in Experimental Diabetes

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Diabetes mellitus is associated with cognitive deficits and neurophysiological and structural changes in the brain. 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. Diabetes was induced with a single injection of alloxan (s.c.). We studied the biochemical and functional changes in the hippocampus and retina of diabetic mice, and the ability of Lutein, a natural antioxidant, to reverse these effects compared to the insulin therapy. The model of diabetes induced by alloxan was used to achieve hyperglycemia. Fourteen days after the alloxan injection, malondialdehyde(MDA) concentration in blood was elevated in diabetic mice. Glutathione-peroxidase (GSH-Px) activity and glutathione (GSH) concentration in hippocampus and eye homogenate without lens (mainly retina) were decreased in diabetic condition, whereas hippocampal and retinal MDA concentrations were higher than controls. Lutein administration (0.2mg/kg p.o.), or insulin treatment(500 mU/g body weight s.c.) on days 4-13, restored MDA levels in serum, hippocampus and retina. Hippocampal and retinal GSH-Px activity, GSH concentration and impaired hippocampal-dependent learning (water maze test) were restored to control values with both treatments.

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Biological activity of lycopene against smoke-induced lung lesions

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Higher intake of lycopene is related to a lower risk of lung cancer in human studies. Lung cancer risk is associated with higher plasma levels of insulin-like growth factor I (IGF-I) and/or lower levels of IGF binding protein-3 (IGFBP-3). However, little is known regarding whether lycopene can inhibit cigarette smoke-induced lung carcinogenesis through modulation of IGF-I/IGFBP-3, cell proliferation, and apoptosis.

We found that ferrets supplemented with lycopene and exposed to smoke had significantly higher plasma IGFBP-3 levels and a lower IGF-I/IGFBP-3 ratio than ferrets exposed to smoke alone. Lycopene supplementation substantially inhibited smoke-induced squamous metaplasia and proliferating cellular nuclear antigen (PCNA) expression in the lungs of ferrets. Furthermore, cigarette smoke exposure greatly increased BAD phosphorylation at both Ser 136 and Ser 112 and significantly decreased cleaved caspase-3 in the lungs of ferrets, as compared with controls. The elevated phosphorylation of BAD and down regulated apoptosis induced by cigarette smoke in the lungs of ferrets was prevented by lycopene supplementation. Lycopene levels were increased in a dose-dependent manner in both plasma and lungs of ferrets supplemented with lycopene alone. However, lycopene levels were markedly lower in both plasma and lungs of ferrets supplemented with lycopene and exposed to smoke. We then investigated whether β-carotene cleaving enzyme, β-carotene-9',10'-monooxygenase which has been cloned from human and mouse tissues, can cleave lycopene at the 9',10' double bond to produce apo-10'-lycopenoid. We cloned a full-length carotene-9',10'-monooxygenase from ferrets, which encodes a protein of 540 amino acids and has 82% identity with human carotene-9',10'-monooxygenase. Using high-performance liquid chromatography analysis, we demonstrate that a homogenate of COS-1 cells overexpressing ferret carotene-9',10'-monooxygenase by transient transfection can cleave both'β-carotene and lycopene at the 9',10' double bond to produce apo-10'-carotenal and apo-10'-lycopenal, respectively. Furthermore,'we have identified apo-10'-lycopenol as one of the metabolites of lycopene in the lungs of ferrets receiving lycopene supplementation. Real time quantitative PCR assay showed that carotene-9',10'-monooxygenase mRNA is expressed in a variety of ferret tissues, including liver, lung and prostate. Interestingly, the expression of carotene-9',10'-monooxygenase in the lungs of ferrets was slightly induced by smoke-exposure but greatly induced by lycopene

supplementation with or without smoke-exposure. Further, the production of apo-10'-lycopenol in the lungs of lycopene-supplemented ferrets was enhanced by smoke-exposure, compared with ferrets supplemented with lycopene alone. In conclusion, lycopene may mediate its protective effects against smoke-induced lung carcinogenesis in ferrets through up-regulating IGFBP-3 and down-regulating phosphorylation of BAD, which promote apoptosis and inhibit cell proliferation. Further, carotene-9',10'-monooxygenase can be induced by lycopene supplementation and can cleave lycopene to form apo-10'-lycopenal in mammals. These studies contribute to our knowledge of lycopene function and metabolism and provide information about the effects of lycopene supplementation and tobacco smoke on lycopene oxidation *in vivo*.

Effects of Carotenoids and Retinoids on Gap Junctional Intercellular Communication

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Stimulation of gap junctional intercellular communication (GJIC) by carotenoids has been discussed as one possible mechanism underlying cancer-preventive properties of carotenoids. Gap junctions are cell-to-cell channels which are formed from connexin proteins, allowing direct exchange of molecules of molecular weight <1 kDa. Gap junctions are involved in transfer of signaling compounds between connected cells. Among the major carotenoids present in human blood and tissues, β-carotene, cryptoxanthin, zeaxanthin and lutein have been found to be efficient inducers of GJIC. It has been shown for canthaxanthin that the effects of carotenoids are at least in part mediated by metabolites or decomposition products like retinoids or apo-carotenoids (1). The central cleavage product of lycopene, acyclo-retinoic acid, showed only a minor effect on GJIC (2) whereas 2,7,11-trimethyl-tetradecahexaene-1,14-dial obtained by chemical oxidation of lycopene was effective (3). Esters of retinoic acid and structural analogs stimulate GJIC at low concentrations, whereas inhibitory effects are found at levels of 10-20 µM. It has been suggested that the underlying mechanism is dependent on the phosphorylation state of connexin43, affecting the intercellular transport of the gap junction protein.

1. Teicher V.B., Kucharski N., Martin H.-D., van der Saag P., Sies H., and Stahl W. Arch Biochem Biophys. 365, 150-155, 1999

2. Stahl W., von Laar J., Martin H.-D., Emmerich T., and Sies H. Arch. Biochem. Biophys. 373, 271-274, 2000

3. Aust O., Ale-Agha N., Zhang L., Wollersen H., Sies H., and Stahl W. Lycopene oxidation product enhances gap junctional communication. Food Chem. Toxicol. 41:1399-407, 2003

Retinol as Regulatory Co-factor for Redox-Activation of Serine/Threonine Kinases

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Nutritional vitamin A depletion has been known for a century to cause a complex deficiency syndrome that affects multiple organs. Lack of retinoic acid (RA) leading to faulty transcription is the best studied mechanism. Several manifestations, including deficiencies in development, growth, reproduction and immunity, were consequences of absence of vitamin A itself, since RA could not fully reverse these symptoms. We and others have described retinoid pathways that operate in the cytoplasm, independently of transcription. We have now identified a likely mechanism of action. Accordingly, vitamin A binds the regulatory domains of serine/threonine kinases (the cRaf and PKC families). Bound vitamin A serves to promote the activation of these kinases by the alternative pathway that operates via reactive oxygen species (ROS) as second messenger. The binding site has been mapped to the zinc-finger domains embedded in the regulatory domains of both PKC and Raf families. Reaction with ROS, assisted by the co-factor, retinol, causes changes in the zinc-fingers that lead to disassembly of the zinc coordination center, relocation of zinc, and a conformational switch. Kinases translocate from cytoplasm to membrane and are enzymatically active. This new paradigm of vitamin A action, that operates in concert with redox mechanisms, may have broad impact on multiple signal chains and illuminate hitherto unexplained vitamin A deficiency syndromes.

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Lycopene and risk of cardiovascular disease

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Cardiovascular disease (CVD) affected approximately 62 million Americans and accounted for about 40 % of total deaths nationwide in 2001. It has been suggested that uncontrolled oxidation, especially of lipoproteins, may induce and/or promote CVD; therefore, moderation of excess oxidation via antioxidants may be a key step in battling CVD. Numerous epidemiological studies describe inverse correlations between the intake of lycopene-containing foods and reduced risk of CVD. Lycopene, a nonprovitamin A carotenoid found in tomatoes, watermelon and certain fruits, has been demonstrated to have the strongest antioxidant capacity of all carotenoids in vitro. Lycopene has been proposed to function through mechanisms of decreased LDL oxidation, inhibition of cholesterol synthesis, increased cell communication and/or enhanced immune function. This paper will review the epidemiological evidence that shows foods containing lycopene may be beneficial in reducing endpoints associated with cardiovascular disease.

Mitochondria as the target for pro-apoptotic synthetic retinoids

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Certain vitamin A analogs and derivatives (retinoids) have shown promising results in preclinical studies and in a few clinical trials of cancer chemoprevention and therapy. Some synthetic retinoids induce apoptosis in vitro and this effect may be important for their clinical potential. We have investigated the mechanism of action of several pro-apoptotic synthetic retinoids including N-(4- hydroxyphenyl)retinamide (4HPR), 6-[3-(1-adamantyl)-4- hydroxyphenyl]-2-naphthalene carboxylic acid (CD437), and [(4- nitrophenyl)amino][2,2,4,4-tetramethyl thiochroman-6-yl)amino] methane-1-thione (SHetA2) using human carcinoma cell lines derived from lung, head and neck, and skin cancers and found that they act primarily on the mitochondria. 4HPR and SHetA2 enhance reactive oxygen species (ROS) generation, and this effect can be suppressed by antioxidants (e.g., butylated hydroxyanisol), which also suppressed apoptosis induction by these retinoids. CD437 did not increase ROS however, all three retinoids suppressed mitochondrial permeability transition and enhanced cytochrome c release from mitochondria to the cytoplasm. Both of these effects were prevented by cyclosporin A, which also decreased apoptosis. The three retinoids increased caspase-3-like activity, and a caspase-3 inhibitor diminished apoptosis. Respiration-deficient clones of cutaneous carcinoma cells generated by chronic exposure of the cells to ethidium bromide to inhibit mitochondrial DNA synthesis exhibited a marked resistance to mitochondrial permeability transition and apoptosis. Several retinoid receptor antagonists failed to prevent apoptosis induction by 4HPR, CD437 and ShetA2. These results demonstrate that these three retinoids are potent apoptosis inducers that act on the mitochondria in carcinoma cells.

New Horizons in Biological Carotenoid and Retinoid Research

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Understanding the role of carotenoids and retinoids in cell and tissue function has reached a more refined level: Noninvasive analytical methods permit examination of molecules in their biological matrix, e.g. in the macula lutea of the eye or in particular areas of the skin. The role of lycopene and its degradation products can be analyzed in terms of their role in the pathogenesis of cancer in particular cells and tissues, e.g. the prostate. These advances are likely to lead to more profound evaluation of the role of these compounds as micronutrients and their significance in health and disease. The interaction with other micronutrients in terms of synergism (or antagonism) is another topic that can be addressed more fruitfully in the future. Discovery of new targets for retinoids is an active field continuing to yield surprises. The role of retinoids in signal transduction and in the modulation of essential processes such as cell cycle control or apoptosis opens novel avenues for pharmacological and medical applications. The field of ligands for various receptors extends from the search of novel molecules by chemical synthesis to the use of biologically occurring retinoid-like compounds, e.g. in research fields of nutrition and phytochemistry.

Posters

Effect of Genistein pre-treatment on differential gene expression induced by oxLDL and homocysteine

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Epidemiological studies indicate that the anti-atherogenic effects of soy consumption are, at least in part, due to isoflavones. In order to study the mechanisms by which genistein, the most abundant soy isoflavone, exerts a protective activity on atherosclerosis, we analyzed the genomic response of HUVEC pre-treated with genistein and exposed to pro-atherogenic stressors. After 24 hours incubation with 2.5 microM genistein, cells were treated either with homocysteine or Cu oxLDL for 12 hours. At the end of treatment cells were harvested, RNA collected and gene expression assessed by a cDNA array technique. Pre-treatment with genistein inverted the trend of expression of a significant proportion of genes that were affected by all treatments. In association with genistein pre-treatment, 32 % of down-regulated genes and 71 % of up-regulated genes by oxLDL, significantly inverted their expression (compared to oxLDL treatment alone) or at least maintained close to their basal control levels. Similarly, 39 % of down-regulated genes and 62 % of up-regulated genes by homocysteine were kept near to the baseline level following genistein pre-treatment or significantly inverted their expression (compared to homocysteine treatment alone). 5 genes affected by genistein pre-treatment (i.e. reverted back near to the baseline or below) in spite the up-regulation induced by oxLDL and homocysteine, encoded for the same proteins. Similarly, 5 genes affected by genistein pre-treatment, i.e. reverted back near to the baseline, or even higher than the baseline, in spite the down-regulation induced by oxLDL and homocysteine, encoded for the same proteins This observation suggests that genistein has a specific effect on cellular response to pro-atherogenic stumuli, possibly independent from its antioxidant capacity.

Antioxidant Activities of Flavonoids from *Nepenthese Gracilis*

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Flavonoids are phytochemicals available in natural sources such as fruits, vegetables and herbs. Recent studies have shown that flavonoids have beneficial effects on numerous diseases such as coronary heart disease, chronic cough, cancers, cataracts, etc. In this study, a tropical pitcher plant Nepenthes gracilis from Singapore, which is traditionally used as a medicine in tropical countries, was chosen. Composition of flavonoids in pitcher leaves was quantified by HPLC and identified by NMR and MS. The major flavonoids isolated from the leaf were flavonol glycosides. To characterize antioxidant profiles and potential health benefits of the pitcher plant, the total free and bound flavonoids were measured by Trolox equivalent antioxidant capacity (TEAC) assay and by the decolorization of the $ABTS^{\cdot+}$. Flavonol glycosides in the pitcher plant showed high antioxidant potency and/or radical scavenging capacity as compared to a well-known antioxidant, Trolox. Specifically, flavonol glycoside gallate esters among isolated flavonol glycoside compounds demonstrated highest antioxidant activities, particularly quercetin 3-O-alpha-(2”-galloylarabinofur anoside) 1.49 ± 0.12 mM and contributed (6 %) (dried weight) to the pitcher leaves. Further studies are needed to better elucidate the biological antioxidant effects of flavonol glycoside gallate ester in vitro and/or in vivo.

Beta-Carotene-induced changes in RARß isoform expression pattern do not influence lung adenoma multiplicity in the NNK-initiated A/J mouse model

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We studied the influence of BC on lung cancer development in the A/J-mouse with NNK as initiator, and lung adenoma multiplicity as functional endpoint. Gene regulation of the putative tumor suppressor RAR beta was determined for each RAR beta isoform, and analyzed for its relevance to predict the endpoint lung cancer. A/J-mice achieved plasma BC levels of up to 3 micromol/L within 4 weeks, and up to 6 micromol/L after 6 months of supplementation on a diet modified to enhance BC absorption. Despite high lung BC concentrations of up to 6 micromol/kg, tumor multiplicity was not significantly affected by the BC treatment, neither in carcinogen-initiated or in uninitiated mice, and irrespective of dose and time point of treatment during cancer formation. Tumor multiplicity was not correlated with BC plasma levels in NNK-treated animals. All RAR beta isoforms were significantly suppressed in adenoma-bearing lungs of NNK-treated animals irrespective of BC treatment. However, the number of tumors per mouse did not correlate with the RAR beta isoform expression levels. BC alone mildly but significantly increased RAR beta 1 and RAR beta 2 and beta 4 after 3 months supplementation. This induction persisted until 6 months for RAR beta 2 and 4. In conclusion, BC-induced change in RAR beta isoform expression pattern was not predictive for tumor multiplicity, but rather indicative for an intact BC metabolism and persistent sensitivity to retinoic acid in the mice.

Peroxynitrite induces an alternative pathway of NF-κB activation in L8 myoblasts through nitration and inhibition of I-κB a degradation.

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Reactive nitrogen species (RNS) are products of reactions between nitric oxide (NO) and reactive oxygen species(ROS).The role of RNS in the activation of the pro-inflammatory transcription factors remains controversial. In this study we attempted to investigate activation of NF-κB by RNS in skeletal muscle cells, and to elucidate the pathways through which this activation proceeds. We demonstrated that RNS alone at micromolar concenrations, are able to activate NF-κB in myoblasts, without involvement of additional stimuli. We also found that RNS can induce not only the classical pathway of NF-κB activation, which involves I-κB degradation, but also an alternative pathway. In this pathway, initiated by peroxynitrite donor SIN-1, degradation of I-κB did not take place in spite of the constant and high levels of NF-κB activity. I-κB underwent nitration, which prevented its phosphorylation that is needed for its ubiquitination and degradation by the proteasome. This finding of I-κB nitration, implies that RNS in myoblasts can activate NF-κB through a novel mechanism that possibly involves I-κB dissociation from NF-κB due to its nitration. The results of this work suggest that not only do RNS alone activate NF-κB in skeletal muscle, but they also can induce an alternative mechanism of NF-κB activation that has not been previously described. The biological implications of this finding are that peroxynitrite can exacerbate inflammatory response mediated by NF-κB.

mtNOS activity: Regulation by substrates and mitochondrial metabolic states

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Nitric oxide (NO) has been recognized as a signaling molecule that acts both as an intercellular messenger and as an intracellular regulator. This molecule is produced in mitochondria by the enzymatic co-oxidation of L-arginine and NADPH by O_2 to yield NADP, L-citrulline and NO, in a reaction catalyzed by a mitochondrial nitric oxide synthase (mtNOS). The aim of this work was to study the regulatory effects of L-arginine, O_2, pH and metabolic states on mtNOS activity from different rat organs: heart, liver, kidney and diaphragm.

The optimum pH for mtNOS activity was in the 6.5-7.4 range for submitochondrial membranes (SMM) of the organs tested, with a NO production of about 0.7-1.4 nmol/min.mg protein. Mitochondria seem to contribute with about 67% (heart), 37% (liver), 30% (kidney) and 24% (diaphragm) of the total cellular NO production. The study of NO production by SMM as a function of L-arginine concentration showed a hyperbolic response. The apparent L-arginine K_M were 36 μM (heart), 70 μM (liver), 4 μM (kidney) and 37 μM (diaphragm). Values for the apparent K_M for O_2 were 3.3 μM (heart), 40 μM (liver), 37 μM (kidney) and 4.6 μM (diaphragm). The state 4/state 3 transition regulates mtNOS activity and NO release in coupled respiring mitochondria. As a whole, NO production rates at state 3 were 40-50% lower than at state 4. The study of the substrate and state 4/state 3 transition dependence of mtNOS activity, provides the basis for the understanding of the physiological relevance of NO production in mitochondria.

Flow Regulation of NADPH Oxidase and Akt Phosphorylation

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INTRODUCTION Shear stress regulates the activity of NADPH oxidase, a major source of superoxide production in vascular endothelial cells. Nox4 is one of the membrane bound NADPH oxidase subunits. The phosphorylation of the signaling molecule Akt activates eNOS, which produces nitric oxide. However, it remains unknown whether pulsatile vs. oscillatory flow conditions regulate the relative expression of Nox4 and Akt phosphorylation. METHODS AND RESULTS Confluent bovine aortic endothelial cells were exposed to pulsatile flow (PF) with a mean shear stress (τ ave) of 25 dyn/cm^2 and oscillating flow (OF) between ± 3 dyn/cm^2 at τ ave of 0. Cell lysates were then analyzed for p-Akt at 15, 30, 60 minutes and for Nox4 and eNOs expression at 4 hours. Real time RT-PCR was performed for Nox4 and eNOs mRNA at 4 hours. In response to OF, Nox4 mRNA was up-regulated by 1.5-fold with a concomitant increase in protein expression, whereas PF down-regulated Nox4 mRNA by 3-fold with a decrease in protein expression ($n=4$, $P<0.05$). OF dephosphorylated Akt by 1.5-fold at 1 hour accompanied by a significant decrease in eNOs mRNA at 4 hours, whereas PF induced p-Akt by 2-fold with an up-regulation of eNOs mRNA by 3-fold ($n=4$, $P<0.05$). CONCLUSION Pulsatile vs. oscillatory flow conditions regulate the relative expression of Nox4 and phosphorylation of Akt, suggesting the important role of shear stress in the production of reactive oxygen and nitrogen species.

Protective role of p53 against oxidative DNA injury

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The p53 tumor suppressor is considered as key guardian of the genome. For a long time it was known that the protective role of p53 is exerted through restriction of growth or survival of abnormal cells. However, accumulating evidenced suggest that in addition to acting as a policeman, the function of p53 involves positive adaptive responses that protect normal cells from potential injury. Presumably, such function of p53 would further contribute to increasing genetic stability and better adaptation to changing environment. We have shown that p53 protects cells against oxidative DNA injury caused by excessive reactive oxygen species (ROS) through transcriptional upregulation of the genes involved in antioxidant defense. p53 mobilizes antioxidant defense even under physiological, non-stressed conditions by activating glutathione peroxidase 1 and members of the newly discovered sestrin gene family, Hi95 and PA26, which are essential regulators of peroxiredoxins. Knocking-down of p53 by siRNA results in accumulation of ROS and increased oxidative injury to DNA. Overexpression of sestrin family members Hi95 and PA26 partially restores the original level of ROS in p53-negative cells and decreases DNA oxidation. Moreover, expression of physiological levels of wild type, but not transcriptionally-inactive mutants of p53 results in decrease in ROS levels. On the contrary, under stress condition the activity of p53 is switched to activation of pro-apoptotic genes that result in massive increase in ROS and apoptosis. The obtained results identify novel protective role of p53, which is attributed to its function under non-stressed conditions.

Reactive Oxygen Species Suppress Hepatitis C Virus RNA Replication In Human Hepatoma Cells

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Hepatitis C virus (HCV) is a positive-stranded RNA virus that causes severe liver diseases, such as cirrhosis and hepatocellular carcinoma. HCV infection is characterized by an increase in the concentrations of reactive oxygen species (ROS), whose effect on HCV replication has yet to be determined. In this report, we investigated the effect of ROS on HCV replication, using a bicistronic subgenomic RNA replicon and a genomic RNA that can replicate in human hepatoma cells. The treatment with peroxide at concentrations that did not deplete intracellular glutathione or induce cell death resulted in significant decreases in the HCV RNA level in the cells. Further studies indicated that such suppressive response to ROS was not due to the suppression of HCV protein synthesis or the destabilization of HCV RNA. Rather, it occurred rapidly at the level of RNA replication. ROS appeared to disrupt active HCV replication complexes, as they reduced the amount of HCV proteins in the Golgi fraction where active HCV RNA replication complexes were found to co-fractionate in the subcellular fractionation experiments. Furthermore, buffering intracellular calcium with BAPTA-AM completely prevented the suppression of HCV RNA replication with ROS. The increased ROS levels in hepatitis C patients may therefore play an important role in the suppression of HCV replication through signaling.

Relative bioavailabilities of coenzyme Q_{10} formulations (ubiquinone and ubiquinol)

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Coenzyme Q_{10} (CoQ_{10}; ubiquinone) belongs to a group of lipophilic quinones found in animals, plants and bacteria. It functions as a mobile electron carrier in the mitochondrial electron transport chain. It is also an antioxidant. Human CoQ_{10} requirements are met by endogenous production as well as by exogenous sources. CoQ_{10} is a popular dietary supplement. Numerous CoQ_{10} containing products are available on the market in the form of compressed tablets (chewable and non-chewable), powder-filled capsules, and liquid-filled softgels. Most of these products contain CoQ_{10} in a form that is very poorly soluble in aqueous media and thus have low bioavailability. A solubilized form of CoQ_{10} (Q-Gel) meeting the USP dissolution criterion was introduced a few years ago that has shown considerably enhanced bioavailability in human subjects (Chopra et al, Int J Vit Nutr Res 1998;68:109-113) and also in animals (Zaghloul et al, Drug Develop Ind Pharm. 2002;28:1195-2000). The reduced form of CoQ_{10} that is stabilized and solubilized (Q-Nol) was developed recently to yield an even more bioavailable form of CoQ_{10} (Miles et al, Nutr Res 2002;22:919-929; Zaghloul et al, Drug Develop Ind Pharm. 2002;28:1195-2000). These findings have been replicated in in-vitro intestinal permeability studies using Caco-2 cells. The superior performance of the solubilized formulations of CoQ_{10} and reduced CoQ_{10} (Q-Gel and Q-Nol) can be attributed to their high dissolution and very small particle size (sub-micron range). Q-Gel and Q-Nol therefore are ideally suited for therapeutic applications requiring high dose CoQ_{10} supplementation such as in the case of mitochondrial cytopathies and neurodegenerative diseases where high plasma CoQ_{10} levels must be achieved to facilitate adequate tissue uptake and crossing of the blood-brain barrier. Preliminary data from clinical trials with Q-Gel and Q-Nol are promising.

Antioxidant and Anticarcinogenic Effects of Selenium

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Selenium, an essential trace element for animals, has been proven to maintain good health and shown to prevent several diseases. White muscle disease and muscular dystrophy have been identified by Se deficient syndrome since 1950. This can be explained by antioxidant characters of selenium as glutathione peroxidase, thioredoxin reductase, phospholipid hydroperoxidase and other selenoproteins such as selenoprotein W and P. Selenium is also needed for the proper functioning of the immune system, and appears to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS. An elevated selenium intake may be associated with reduced risk. Se supplementation has been dramatically reduced cancer incidence, such as prostate, colon, lung and pancreatic cancers in human. It has been known that the inhibition of cancer cell proliferation induce cell cycle arrest and apoptosis. We demonstrated that treatment with selenome thylselenocysteine induced apoptosis in HL-60 cells via increased reactive oxygen species. Apoptosis was detected by sequencial events, mitochondrial membrane potential, cytochrome C release, and caspase activation. Treatment with lower selenite (λ-3 μM) in HT1080 cells inhibited tumor cell invasion by abolishing the activation of matrix metalloproteinse-2 and -9 and urokinase type plasminogen activation and reduced expression their mRNA, which were related to reduce activation of transcriptional factor such as AP-1 and NF-κB. These results suggest that moderate concentration of Se induces apoptosis, and lower concentration is effective to inhibit tumor invasion and further possible metastasis.

Carotenoids as Antioxidants for the Prevention of Diabetic Complications

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In Diabetes Mellitus, two important reactions, i.e., Glycation (Maillard reaction) and lipid peroxidation occur. These two reactions are considered to be the causative factors for the complications of diabetic patients in the vascular system, skin, kidney, retina, peripheral nerves and the lens. In our studies, we found that Carotenoids such as Lutein, Astaxanthin and Lycopene are very effective for the prevention of diabetic complications.When these carotenoids were mixed with Tocopherol or Proanthocyanidin or Sesamin, their antioxidative effects were increased. Chlorella (carotenoids, especially Lutein–rich green alga), was used as source of Lutein. Asthaxanthin extracted from krills was used as source of Asthaxanthin. Lycopene extracted from tomatoes was used as Lycopene source. STZ diabetic Wistar rats or SAM-P8 (Senescence Accelerated Mice) were used in our experiments. Then, the following were made clear: 1) Inhibition of cataract formation was observed for astaxanthin, lutein, and lycopene. 2) Astaxanthin increased the survival rate of diabetic SAM-P8. 3)Strong inhibition of lipid peroxidation and glycohemoglobin A1c formation were observed for astaxanthin and lutein. 4) Inhibition of oxidation of fatty acids was also observed. 5) Inhibition of the level of lipid peroxidation in the lens was very clear in the case of asthaxanthin and lutein. 6) Asthaxanthi increased the survival rate of diabetic SAM-P8. From these results, it is estimated that lipid peroxidation is an important cause of cataract, and dietary carotenoids are effective for the prevention of diabetic complication.

References:

[1]Nguyen VC et al., J. Nutr.Sci.Vitaminol., 49,334-339(2003);

[2]Nguyen VC et al., Japan Patent applied No 256207(2000); Nguyen VC et al., Japan Patent applied No 351766(1999); Nguyen VC et al., Japan Patent applied No 276721 (1998).

Fast And Sensitive Determination Of Total F2-α-isoprostanes In Plasma With HPLC-Tandem-MS

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The oxidative stress hypothesis proposes that reactive oxygen and reactive nitrogen species are involved in the pathogenesis of several chronic diseases such as cancer, cardiovascular disease, ageing and age-related disease. Epidemiological studies have consistently demonstrated an association between fruit and vegetable consumption and a reduced risk of oxidative stress-mediated diseases. The underlying hypothesis suggests that antioxidants such as vitamin C, carotenoids, flavonoids may be responsible for the beneficial effects. Chemically, oxidative damage to tissue macromolecules (DNA, fat, protein) occurs and many reaction and breakdown products have been identified. In order to assess the efficacy of food and dietary ingredients in intervention studies with humans, suitable biomarkers need to be identified. Recently, an extensive and critical review has been written on this (Griffiths et al., Mol. Aspects. Med. 1-3: 101-208), in which F2-isoprostanes were identified as one of the most promising biomarkers available. However, the practical analysis of F2-isoprostanes is technically complicated. ELISA assays are available but these inherently have the drawback of cross-reactivity. As an alternative, many laboratories use the laborious GC-MS method that requires a difficult and tedious sample preparation step. Recently LC-MS and LC-MS-MS have become available in many laboratories, in theory leading to easier sample preparation methods for the determination of analytes in biological fluids. For this purpose a sensitive and highly selective HPLC-Tandem MS method is developed for the analyses of F2-α-isoprostanes in plasma. The recovery was 98%, the intermediate precision SI(T) was 9%, and the LOQ was 6 pg/ml.

Prolactin in the Lung: Modulation of Nitric Oxide During Inflammation

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Prolactin (PRL), the pituitary hormone originally associated with milk secretion, has been implicated as a modulator of immune function, and some of its actions may be linked to nitric oxide (NO) synthesis. We hypothesized that PRL can modulate NO synthesis by pulmonary cells during acute inflammation. To test this hypothesis *in vitro* and *in vivo* experiments were performed. *In vitro:* The expression of PRL receptor mRNA (PRLR) was demonstrated in non-stimulated rat alveolar type II epithelial cells and in cytokine-stimulated pulmonary fibroblasts. PRL inhibited cytokine-induced NO production and the expression of the inducible NO synthase (iNOS) by fibroblasts. *In vivo:* We assessed the expression of PRLR mRNA in lung tissues from C57Bl/6 mice subjected to intranasal instillation of lypopolysaccharides. Inflammation was evaluated by measurement of the total cell/granulocyte number, NO metabolites and cytokine concentration in the bronchoalveolar lavage fluids. We show that during the course of inflammation PRLR is down-regulated at the time when NO metabolites and pro-inflammatory cytokines are elevated. The down-regulation of PRLR may serve as a mechanism to block PRL inhibitory signal on iNOS expression, thus facilitating the cytokine-induced NO synthesis during inflammation in vivo. These results suggest previously unrecognized mechanisms by which PRL and PRLR may play significant modulatory roles in immune-inflammatory processes.

A Diet Supplemented With 20% Of Biscuits Enriched In Antioxidants, For 5 Or 15 Weeks, Improves Leucocyte Functions In Prematurely-aging Mice

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Aging is associated with a decline in the immune functions which is linked to a progresive oxidative stress. Antioxidant defences of immune cells play a vital role in maintaining immune cell functions. We studied the effect of a diet supplemented with biscuits enriched in antioxidants (vit. C, vit. E, beta-carotene, Zn and Se), ingested for 5 or 15 weeks vs control, on several functions of leucocytes from prematurely aging mice (PAM). Swiss CD1 mice, 22 weeks old, were used. PAM were detected after the mice carried out an exploratory test in a T-shaped maze. Peritoneal suspensions, containing the main immune system cells, were obtained and their functions were studied as follows: In macrophages the different steps of the phagocytic process (adherence to tissues, chemotaxis, phagocytosis capacity, and intracellular production of superoxide anion and hydrogen peroxide) were assessed. In lymphocytes, the adherence, chemotaxis, proliferative response to ConA and the release of interleukin 2 were determined. The natural killer cell activity was also analized. Results show that PAM have the immune function values studied at the levels of older animals (which have lower values than NPAM in all functions with the exception of adherence, which is higher in PAM, as it occurs in aged animals). Both 5 and 15 weeks of supplementation increased the immune parameters that were decreased in PAM and decreased the adherence capacity. In conclusion, since the functionality of the immune cells is a marker of health and longevity predictor, the supplementation used in the present work could be useful for maintenence of subject health and resulting functional longevity.

The Role Of Glutaredoxin Dethiolase Activity Upon The Redox Regulation Of The 20s Proteasome From *S. Cerevisiae*

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We have previously demonstrated that the chymotrypsin-like activity of the 20S proteasome core purified from *S. cerevisiae* is affected by redox modification of its Cys residues (Cys-SH). Those residues are oxidized to Cys-sulfenic acid (Cys-SOH) followed by S-glutathionylation (Cys-SSG) by reduced glutathione. This mechanism has been described for other proteins and thereafter deglutathionylation occurs through the so-called dethiolase glutaredoxin activity, as attested by some few examples in the literature. In the present work we describe that recombinant yeast glutaredoxin2 is able to release glutathione from *in vitro* S-glutathionylated 20S proteasome core followed by recovering of chymotrypsin-like activity. Moreover, ascorbate was able to partly reduce oxidized forms of Cys-proteasome residues, most probably the –Cys-SOH form, concomitantly to partial rescue of chymotrypsin-like activity. Experiments performed *in vivo* revealed that the chymotrypsin-like specific activity of 20S proteasome isolated from cells grown in glycerol-containing medium is 5-fold the specific activity found in preparations of 20S proteasome isolated from cells grown in glucose-containing medium. These findings were positively correlated to the intracellular reductive capacity evaluated by the GSH/GSSG ratio and to glutaredoxin2 expression. Results reported are in agreement with our early hypothesis that proteasomal activity is regulated by redox modifications.

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Thiol Antioxidants Induce Detoxication Enzymes in Mouse Midbrain

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Detoxication of dopamine-derived quinones may provide neuroprotection in Parkinson's disease (PD). The present study analyzed the ability of, the thiol antioxidants α-lipoic acid (LA) and 1,2-dithiole-3-thione (D3T) to induce detoxication enzymes in the mouse midbrain. Mice were injected ip with D3T, LA daily for 1, 2 or 4 days, and decapitated 24 h after the last injection. Liver and midbrain were processed for quantitative PCR-based detection of gene expression or spectrophotometric analysis of NADP(H)Quinone oxidoreductase (NQO1) enzyme activity. 1 Day of D3T treatment strongly stimulated NQO1, Glutathione S-Transferases (GST) GSTμ and GSTα gene expression in the liver and, to a lesser extent, yet significantly, in the midbrain. In addition, NQO1 enzyme activity was increased in liver but not midbrain samples after 2 and 4 days. The effects of LA were limited to an increase of GSTα expression in liver and mild elevation of GSTμ expression in midbrain after 1 day. The study shows, for the first time, that D3T induces multiple DA quinone detoxication enzymes in midbrain whereas LA is less effective in this respect. Given that the D3T-induced effects are mediated by activation of the Nrf2-ARE signaling system, which regulates the expression of a wide range of detoxication and anti-oxidant enzymes, dithiolethiones with the capacity to stimulate this pathway may provide a potent tool for neuroprotection in PD.

SOD1 Gene Expression Is Modulated By DSCR1(adapt78)

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SOD1 (intracellular Cu,Zn superoxide dismutase) is a major component of cellular protection against oxidative stress. Previous studies, however, have indicated that SOD1 might either protect or damage cells, depending on its levels. Although the SOD1 gene is well characterized, little is know about its regulation. Here, using regulated expression of the DSCR1(Adapt78) gene in PC-12 cells, followed by microarray analysis of mRNAs from these cells, we have discovered that SOD1 expression is modulated by DSCR1(Adapt78). We further confirmed that DSCR1(Adapt78) expression stimulates SOD1 protein production and leads to increased sod 1 activity. Thus, for the first time, we have demonstrated an upstream element involved in SOD1 regulation. DSCR1(Adapt78) is a gene induced by multiple stresses and we propose a model in which stress induces the SOD1 gene through DSCR1(Adapt78). These data may be important for understanding mechanisms of cell protection against oxidative stress and for our understanding of human pathologies associated with abnormal SOD1 levels, such as Downs' syndrome.

Vitamin C Transporters and Ascorbate-stimulated Chloride Transport in Airways

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Vitamin C (L-ascorbate) is present in the thin layer of fluid that lines the respiratory tract. Local vitamin C deficiencies have been reported in asthma, cystic fibrosis, chronic obstructive pulmonary disease and smokers. Chloride transport function is important for normal mucociliary clearance. We focused on the cystic fibrosis transmembrane conductance regulator (CFTR), a cyclic AMP-dependent Cl channel and key modulator of the fluidity of the airway surface liquid. Vitamin C opened CFTR channels by increasing the average open probability from 0 to 0.21 ± 0.08. Exposure to vitamin C stimulated transepithelial Cl secretion to 68% of the maximal cAMP-stimulated Cl currents with a half-maximal stimulatory constant of 36.5 ± 2.9 mM, which corresponds to physiological concentrations. In vivo efficacy was evaluated using the nasal PD assay. Sodium-dependency and phloretin-sensitivity, as well as localization of SVCT2 to the apical membrane pole, support a model where an apical vitamin C transporter is central for relaying the effect of ascorbate to CFTR. HPLC revealed a time-dependent uptake of vitamin C into airway epithelia, and approximately 20% of its cellular entry occurred within the first 3 minutes. Following incubation with ascorbate for 1 hour, the final ascorbate concentration of well-differentiated human airway epithelia increased from 0.3 to 8.7 nmoles/mg protein (100 µM ascorbate) or to 29.1 nmoles/mg protein (1 µM ascorbate). We conclude that vitamin C, in addition to its well-known properties as an antioxidant, functions as a biological regulator of CFTR. The pool of vitamin C in the airways represents a potential nutraceutical and pharmaceutical target. Supported by NIH (HL071829, AT002034), CFF (ILLEK02G0), and CFRI.

Topology of ONOO- production by mitochondria

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ONOO- is formed from the reaction of NO with $O_2^{-\bullet}$ at diffusion control rates. The formation of ONOO- by mitochondria is dependent on 1) the rate and topologic distribution of $O_2^{-\bullet}$ and 2) localization of superoxide dismutase (SOD), which competes with NO for $O_2^{-\bullet}$ $O_2^{-\bullet}$, generated by the electron transfer chain, is released towards the matrix and intermembrane space. While the intermembrane lacks measurable SOD activity, the matrix contains an effective Mn-SOD. Thus, ONOO- formation would be favored in the intermembrane space due to lack of competition by SOD at physiological NO levels. EPR analysis of mitoplasts treated with DETA-NO (1 μM steady-state levels of NO) resulted in the formation of a DMPO-OH adduct. The EPR spectra were competitively inhibited by SOD and DMSO, suggesting ONOO- formation in the intermembrane space. Measurements with a NO electrode show NO consumption at a rate of 12 nmols/mg/min by brain mitoplast when treated with 1uM steady-state NO. Addition of exogenous Cu,Zn-SOD was found to inhibit this NO consumption in a dose dependent manner, with a maximum of 45% inhibition. Pathways of NO consumption by mitoplasts, include ONOO- formation, reactions with ubisemiquinone, and binding to cytochrome oxidase. Reactions of NO with ubisemiquinone and cytochrome oxidase have been estimated to be responsible for 40% of NO consumption by mitochondria. Since exogenous SOD was able to inhibit a large percent of NO consumed to form ONOO-, our results indicate that a significant portion of ONOO- generated in mitochondria is formed in the intermembrane space compartment.

European Research on the Functional Effects of Dietary Antioxidants (Project EUROFEDA): Biomarkers

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Oxidative biomarkers have been used to evaluate the efficacy of antioxidants *in vitro*, *ex vivo* and *in vivo*. Mixed results have been obtained in studies using biomarkers, functional endpoints and disease process. The importance of biomarkers and the development of disease, is highlighted by recent antioxidant intervention studies that have not demonstrated benefit using disease activity as an outcome measure after up to 5 years. In contrast, shorter-term intervention studies have identified antioxidant benefit using biomarkers. Complicating phenomena underlying these mixed observations of antioxidant effect are: validity of the biomarker, variation in study design, degree of oxidative load. For any extrapolations from biomarker to free radical effect and disease to be valid, one must be entirely confident in both the chemical specificity of biomarker formation and also the validity of our methods of measurement. We have undertaken a comprehensive review of the methodology currently adopted worldwide for the measurements of biomarkers derived from protein, lipid and DNA, together with evaluation of measures of oxidant/antioxidant status (Mol. Aspects Med., 23 (2002) 209-285). We have examined the validation status of biomarker assays, summarised the evidence for their relationship to disease and have presented the evidence for modulation of biomarker levels by dietary antioxidants.

Selenoproteins And Fish Fertility

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In this study, we determined the selenoproteins enzymatic activities in a lower vertebrate, the male of Dicentrarchus labrax. Our enzymatic evaluation of PhGPx provided evidence in fish, whereas testis occurrence of GPx as fish antioxidants, reinforces our previous biochemical studies [1]. Our data showed GPx and PhGPx differently related to the sea bass testis. GPx activity does not suggest any specific role for testicular function. In fact, its expression and activity are unchanged during sexual maturation of sperms. In contrast, PhGPx is expressed only in the recrudescence of the gonadal activity, and it might be involved in the process of spermatogenesis [2]. The peculiar expression pattern of PhGPx in fish results similar to the mammalian one's [3]. The tissue distribution of PhGPx activity obtained in fish, being low in the liver and heart but high in the testis, may be related to the antioxidant demand [4]. In opposite, the GPx activity expressed in testis at lower levels respect to control tissues may be implicated in the metabolism of H_2O_2 associated with steroid hormone synthesis in Leydig cells [5]. Since selenoproteins are essential for the proper development and fertilizing capacity and the oxidative stress has been demonstrated to play a key role in male infertility [6], our goal will be to identify methods that will help to measure it with accuracy.

References: [1] G.GUERRIERO et al. Comp. Biochem. Physiol. Part A 132, 205-211, 2002. [2] G.GUERRIERO et al. International Proceeding Monduzzi, Bologna (in press.). [3] A.ROVERI et al. J. Biol. Chem. 267, 6142–614, 1992. [4] L.ZHANG et al. Biochem. Biophys. Acta 1006, 140–143, 1989. [5] X.G.LEI et al. J. Nutr. 125, 1438–1446, 1995. [6] M.MAIORINO AND F.URSINI. Biol.Chem. 383, 591-597, 2002.

Free radicals scavenging activity of hydroxyflavone sodium salts comparing to hydroxyflavones themselves activity

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Objectives: Different studies have demonstrated that the bioactivities of hydroxyflavones are due to their antioxidant and free radical scavenging activity. Nowadays, most interest has been devoted to structure-activity relationships; but, the main problems encountered in these studies are the low solubility of some hydroxyflavones in aqueous solution and the pro-oxidant character of the hydroxyl group in position 3. Methodology: In the present investigation, we resolved these problems by preparing the corresponding hydroxyflavone sodium salts. In this way, we studied the hydroxyl free radical scavenging activity of different hydroxyflavone salts by using the Fenton reaction model, and the superoxide scavenging activity by using the xanthine oxydase model.

Results: The results obtained show clearly that the hydroxyl radical scavenging activity of the hydroxyflavone salt increases at least two times comparing to the corresponding hydroxyflavone itself. As considering the superoxide scavenging activity, the salt of hydroxyflavone is as good as the corresponding hydroxyflavone. Moreover we noticed that for a good scavenging activity, the hydroxyl in 3' position must be free, only the hydroxyl groups of 3 and 4' are required substituted with sodium, when the sodium salt of 7-hydroxyl group of the hydroxyflavone did not have an important implication in radical scavenging.

Conclusion: The salt forms of hydroxyflavones are interesting free radical scavenger compounds which present a hydrophilic character.

FeEDTA, a Redox Model For Heme Protein-mediated LDL Nitration

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Protein tyrosine nitration is increased in atherosclerotic lesions. Heme proteins such as myeloperoxidase and hemoglobin can localize in atherosclerotic lesions. Since transition metals and heme can catalyze oxidation and nitration reactions it was of interest to study the differential effects of chelated (FeEDTA) and free iron (Fe) on LDL nitration/oxidation by peroxynitrite (PN). Both FeEDTA and free Fe dose-dependently catalyzed the formation of nitrotyrosine (NT) in the presence of PN. FeEDTA had significantly higher nitrating potential, catalyzing NT formation at a two fold greater rate than free Fe. FeEDTA and free Fe catalyzed LDL lipid peroxidation (LP) measured as TBARS, and chelated iron was also more effective at catalyzing LP. Both FeEDTA and Fe catalyzed degradation of PN. Analysis of PN degradation showed significantly higher rates with FeEDTA compared to that of free Fe. Thus, generation of PN degradation intermediates is critical for LDL nitration/oxidation. The rate of NO_2^- formation strongly correlated with the NT levels and was higher for FeEDTA at concentrations above 10μM. Kinetic analysis demonstrated that $NO_2^\cdot$ is formed faster but degraded slower for FeEDTA suggesting a higher steady state for nitrating intermediates in the presence of chelated iron. Our data suggest FeEDTA as a redox model for heme proteins in nitration reactions.

Usnic acid induced necrosis of cultured hepatocytes: inhibition of mitochondrial function and oxidative stress

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Usnic acid, a lichen acid, is a compound found in crude medicines and dietary supplements including Lipokinetix, a supplement marketed as a weight loss agent that caused hepatotoxicity and acute liver failure in patients. We examined the toxicity of usnic acid and assessed whether usnic acid may be contributing to hepatotoxicity caused by Lipokinetix. In primary cultured murine hepatocytes, usnic acid treatment (5 uM) resulted in 98% percent necrosis within 16 hours (no apoptosis was detected). Usnic acid inhibited mitochondria respiration and caused a drop in ATP levels in cultured hepatocytes. Oxidative stress appears to be central in usnic acid-induced hepatotoxicity based on the following findings: 1) antioxidants (BHT + vitamin E) decreased usnic acid-induced necrosis by nearly 70%; 2) depletion of mitochondrial GSH with diethylmaleate increased susceptibility of hepatocytes to usnic acid; 3) usnic acid treatment was associated with increase free radical generation, measured using the fluorescent probe, DCFH. The source of reactive oxygen species after usnic acid treatment include autoxidation of usnic acid and increased hydrogen peroxide generation by mitochondria caused by usnic acid inhibition of the respiratory chain, with the latter playing a more prominent role. Taken together, our results suggest that usnic acid is a strong hepatotoxic agent that triggers oxidative stress and disrupts the normal metabolic processes of cells. Usnic acid therefore may contribute to the hepatotoxic effects of Lipokinetix and its use in any supplement must come into question.

Effect of glutathione depletion on hydrogen peroxide production from mitochondria

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Mitochondrial GSH depletion occurs in several pathological conditions including chronic ethanol intake, cerebral ischemia, and during liver regeneration. The physiological consequence of moderate mitochondrial GSH depletion seen in vivo in these pathological situations is not known. In this study, mitochondrial O_2 and H_2O_2 generation and their interplay with matrix GSH were examined in isolated mitochondria. GSH was found to modulate H_2O_2 production from the matrix but not O_2 generation from the intermembrane space. Low levels of GSH depletion (from 0-40%, depending on the rate of H_2O_2 production) had no effect on H_2O_2 diffusion from mitochondria. Once this GSH depletion threshold was reached, GSH loss corresponded to a linear increase in H_2O_2 production by mitochondria. The impact of 50% mitochondrial GSH depletion, as seen in certain pathological conditions in vivo, on H_2O_2 production by mitochondria depended on the metabolic state of mitochondria, which governs its rate of H_2O_2 production. The greater the rate of H_2O_2 generation the greater the effect 50% GSH depletion had on enhancing H_2O_2 production. Because basal levels of H_2O_2 are very low and mitochondrial GSH levels are very high, even 50% mitochondrial GSH depletion may not have a big impact on H_2O_2 production by mitochondria in vivo under normal circumstances. But mitochondria can undergo fluxes in H_2O_2 production, and during times of high H_2O_2 production, loss of mitochondrial GSH may magnify H_2O_2 production by mitochondria and enhance oxidative damage to cells.

Sites and topology of superoxide production by mitochondria

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O_2 production by mitochondria has been suggested to play a pathological role in aging, Parkinson's disease, and numerous other diseases. In this work, the sites and topology of O_2 production was examined in brain and heart mitochondria. Complex I was observed to generate O_2 predominately towards the matrix. The production of O_2 by complex I occurred during reverse electron flow utilizing succinate, or with rotenone in the presence of complex I substrates. Complex III was observed to generate O_2 towards the matrix and/or the intermembrane space, depending on the type of complex III inhibitor used. Treatment of mitochondria with antimycin, resulted in O_2 production both toward the matrix and intermembrane space, while myxothizol treatment resulted in O2.- generation only towards the matrix. Myxothizol could also inhibit the antimycin-induced O_2 production towards the intermembrane space. Stigmatellin treatment resulted in bursts of O_2 both towards the matrix and intermembrane space, but to a lesser extent than antimycin. Inhibition of complex IV by KCN also resulted in production of O_2 both towards the matrix and intermembrane space. This generation of O_2 by complex IV inhibition is likely to occur because of a back flow of electrons at complex III. Our results suggests the direction of O_2 production along the inner membrane depends on the type of inhibition that occurs. The topology of O_2 production should be taken in account to understand the pathological role mitochondrial O_2 has in various diseases.

Sites and mechanisms of aconitase inactivation by peroxynitrite: Modulation by citrate and glutathione

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Aconitase, a Krebs cycle enzyme, contains a Fe-S cluster in the active site and cysteine residues that may be targets of reactive molecules. The sites and mechanisms of aconitase inactivation by ONOO- were investigated using purified aconitase. ONOO- was found to inactivate aconitase in a dose dependent manner, with only ~ 3 μM of ONOO- being needed for a 50% inhibition. The presence of the substrate, citrate, shifts the dose curve and higher than 200 uM of ONOO- was needed to inhibit aconitase by 50%. EPR analysis showed that low levels of ONOO- oxidized the active $[4Fe\text{-}4S]^{2+}$ cluster into the inactive $[3Fe\text{-}4S]^{1+}$ form. The oxidation of the Fe-S cluster by ONOO- was completely protected by citrate. The inactivation of aconitase by high levels of ONOO- with citrate present was due to ONOO- oxidation of thiols distal to the active site. EPR analysis with DMPO, showed ONOO– treatment to aconitase caused the formation an immobilized DMPO/• cysteinyl-aconitase adduct, that was not inhibitable by citrate. GSH, a major scavenger of ONOO- in the matrix, surprisingly enhanced ONOO- damage to aconitase by 20%. EPR analysis suggests that glutathionyl radical, generated between the reaction of GSH and ONOO-, was reacting with aconitase. GSSG was also found to inhibit aconitase activity by 45%. DTT reversed the GSSG-induced inactivation of aconitase, thus suggesting glutathionylation of aconitase. It may be surmised that both the metabolic and thiol redox state of the mitochondrial matrix is important in determining aconitase functionality.

Expression of DSCR1(adapt78) Isoforms in Alzheimer's Disease

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DSCR1(Adapt78) was identified by our laboratory during differential display studies, as a gene strongly upregulated in mammalian cells during transient adaptation to oxidative stress. Our work has shown significant levels of DSCR1 mRNA in normal adult human brain, and chronic overexpression in brain areas affected by Alzheimer disease. DSCR1(Adapt78) may be protective against transient oxidative stress, but damaging when chronically expressed, as in Alzheimer disease. The DSCR1(Adapt78) protein product, calcipressin1, is an inhibitor of calcineurin, a major brain phosphatase. Adaptation to oxidative stress appears to be partly due to calcineurin inhibition by transiently overexpressed calcipressin1. DSCR1(Adapt78) contains seven exons that are alternatively spliced. We found that two major isoforms are expressed in human brain: isoform 1, containing exons 1, 5,6 and 7; and isoform 4, containing exons 4, 5, 6 and 7. We examined the expression of each isoform in (post mortem) human brains and found both mRNA isoforms expressed predominantly in neuronal cells; using combined in situ hybridization and immunocytochemistry. We further determined that both isoforms of calcipressin1 are expressed in adult human brain, using exon 1 and exon 4 antibodies, and expression of calcipressin1 isoform 1 is greater in brain regions affected by Alzheimer disease, while isoform 4 does not appear altered in Alzheimer's. Thus, Alzheimer disease may involve a crucial increase in expression of the DSCR1(Adapt78) gene product, calcipressin1-1 in brain regions affected by the disease.

17β-Estradiol Attenuates Oscillatory Flow-Induced LDL Oxidation

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17β-Estradiol(E2) is reported to confer cardiovascular protection, particularly in postmenopausal women. Oscillatory flow (OF) shear stress, which occurs at the arterial bifurcation (sites of atherosclerosis predilection), induces atherogenic responses in endothelial cells (EC). We tested whether E2 reduced the OF-induced expression of NADPH oxidase (Nox4) and LDL oxidation, since increased Nox-4 expression occurs with OF. Methods and Results- Confluent bovine aortic endothelial cells (EC) monolayers were incubated in the presence and absence of E2 (5 nM) and exposed to OF conditions. Culture media were analyzed for O_2^- production at 1, 2, 3, and 4 hours. Real-time RT-PCR for Nox4, and endothelial nitric oxide synthase (eNOs) was normalized to β-actin levels. The effects of OF were compared to cultures maintained under static conditions (controls). OF increased Nox4 mRNA expression by 3.4-fold ± 0.7 and the rate of O_2^- production by 3.0-fold ± 0.4. In the presence of E2, Nox4 mRNA expression and rates of O_2^- production were significantly decreased by 1.4-fold ± 0.4 and 3-fold ± 0.6. In response to OF, the extent of LDL oxidative modification increased by 67 ± 17 % relative to controls and was accompanied by a decrease in eNOS expression. E2 attenuated the extent of LDL modification (51 ± 12) with an accompanying increase in (eNOs) expression. Conclusions: Oscillatory flow-induced LDL oxidation is attenuated in the presence of E2 and formation of modified LDL occurs via increased reactive species production as determined by the relative activities of Nox-4 and eNOS.

Induction of NADPH Oxidase Subunit, Nox4, by Ox-PAPC: Implications of NAD(P)H Autofluorescence and MMP Expression

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Oxidized-1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (ox-PAPC) is a biologically active component of minimally modified LDL. We tested whether ox-PAPC activates NADPH oxidase subunit, Nox4, and whether increased $O_2^{-\bullet}$ production via NADPH utilization activates MMP-2. Bovine aortic endothelial cells (BAEC) were treated in two culture media: (1) DMEM vs. (2) glucose-free DMEM medium plus 10μM of 2-Deoxy-Glucose (2-DOG), the latter being an anti-metabolite that blocks NADPH production by the pentose shunt. After 12 hours, cultures were incubated with 50 microg/ml ox-PAPC for 4 hours. Ox-PAPC increased Nox4 mRNA expression by 2.4-fold +/- 0.1 and the rate of $O_2^{-\bullet}$ production, whereas in 2-DOG medium, the rate of $O_2^{-\bullet}$ production decreased (DMEM=103.7+/-10.8 vs. 2-DOG = -20±9.8 nM/min/million cells, $P<0.05$, n=5). The intensity of NAD(P)H autofluorescence decreased by 28±12% in BAEC incubated with ox-PAPC compared to untreated cells. In the presence of ox-PAPC, MMP-2 activities were increased by 1.5-fold. Ox-PAPC stimulates an NADPH-dependent production of ROS, implicating ox-PAPC as a mediator of MMP activation in endothelium. In the presence of Ox-PAPC, NAD(P)H autofluorescent intensities reflect the in NADPH oxidase-dependent $O_2^{-\bullet}$ production by ox-PAPC.

Glutathione Synthesis in *Streptococcus agalactiae*: One Protein Accounts for γ-Glutamylcysteine Synthetase and Glutathione Synthetase Activities

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Glutathione (GSH) is the main non-protein thiol in many Gram-negative bacteria and most eukaryotes. In all cases examined to date, GSH is synthesized by the sequential action of γ -glutamylcysteine synthetase (γ-GCS, Reaction 1) and GSH synthetase (Reaction 2); a separate gene codes each enzyme.

Reaction 1: Glutamate + Cysteine + ATP → γ -Glutamylcysteine + ADP + Pi

Reaction 2: γ -Glutamylcysteine + Glycine + ATP → GSH + ADP + Pi

Endogenous GSH synthesis is uncommon among the Gram-positive bacteria, and, to date, neither the genes nor the proteins involved have been clearly identified. In the present report we show that crude extracts of *Streptococcus agalactiae* are able to synthesize both γ-glutamylcysteine and GSH. We identified the putative gene for *S. agalactiae* γ-GCS, and have cloned, expressed and purified to near homogeneity the corresponding native and poly-His tagged γ-GCS enzyme. Surprisingly, it was discovered that the isolated protein catalyzes both the γ-GCS reaction (specific activity ~900 units/mg) and the GSH synthetase reaction (specific activity ~700 units/mg). The isolated protein is the first enzyme of GSH synthesis identified in a Gram-positive organism and is the first bifunctional γ-GCS – GSH synthetase reported. A similar gene occurs in several other, mostly Gram-positive, bacteria, suggesting a new family of GSH synthesis enzymes. The implications of these results will

Decrease Of The Oxidative Stress In Leukocytes From Adult Mice Showing Premature Aging By A Diet Supplemented With 5% Or 20% Of Biscuits Enriched In Antioxidants

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With aging there is an increase of oxidative stress due to a higher oxidant production and lower levels of antioxidant defences. The oxidant/antioxidant balance in leukocytes is essential for their functions. Mice classified as PAM (prematurely ageing mice) show an early decline of several nervous and immune functions in comparison to control mice or NPAM (non-prematurely ageing mice) of the same chronological age. In the present work we have studied the effect of a diet supplemented with two different amounts (low = 5% and high 20 % w/w) of biscuits enriched in antioxidants (vitamin C, vitamin E, β-carotene, zinc and selenium) vs control (AIN-93A diet) on several parameters of oxidative stress in leucocytes from adult PAM. Adult female ICR (CD1) mice classified as PAM at 7 months of age were supplemented during 5 weeks and their peritoneal leukocytes were analyzed. Results show that oxidation/inflammation parameters: extracelular superoxide anion, GSSG, GSSG/GSH, TNFα and PGE2 are higher in cells from PAM than in those from NPAM. Conversely, catalase activity and GSH levels are lower in leukocytes from PAM than in those from NPAM. Low antioxidant diet already decreases the oxidative stress of leucocytes from PAM, increasing scavenger enzyme activities and GSH and reducing extracelular superoxide anion, GSSG, GSSG/GSH. High antioxidant diet induces an decrease of all oxidant parameters and a decrease of all antioxidants. In conclusion, the ingestion for 5 weeks of a diet enriched in antioxidants decreases oxidative stress. The present findings confirm the important role of antioxidants for health preservation and resulting functional longevity.

A New Liquid Chromatography/Mass-Spectrometry Method for a Comprehensive Assessment of Sulfur Amino Acid Oxidation States in Biological Samples

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Oxidative stress is a contributing cause in numerous chronic illnesses. Oxidants can damage biological macromolecules and disrupt cellular homeostasis. Levels of Aminothiols, first line of defense against various types of oxidants, decline during various human disease states, due in part to their oxidation to disulfides. Changes in oxidation states of glutathione (GSH) have primarily been used as a measure of the intracellular thiol redox state. Focus on GSH is partly due to technical constraints that limit the sensitivity of detection of other less abundant aminothiols. We developed a sensitive liquid chromatography/tandem mass-spectrometry (LC/MS/MS) method for detecting and quantifying oxidation states of a comprehensive panel of aminothiols. A strong cation exchange, solid phase extraction procedure is used to concentrate the acid-soluble aminothiols in biological samples. Isopropylchloroformate (IPCF) is added to rapidly (2-3 min) esterify the reactive sulfhyrdryl, carboxyl, and amino groups. IPCF modifies the free thiol groups at low pH ranges (1-6) and provides a more precise determination of thiol redox states. The use of IPCF, combined with LC/MS/MS greatly improves the detection sensitivity and selectivity of aminothiols. The detailed redox profiles of aminothiols (e.g., homocysteine, cysteine, cysteinylglycine, and glutathione) are readily obtained by this new method. Moreover, the link between antioxidant redox changes and oxidative protein damage can be quantified by simultaneously measuring methionine sulfoxide, which is product of methionine oxidation. The analysis of a comprehensive aminothiol panel made possible by this method will aid research into the metabolic interactions among these aminothiols under normal and pathophysiological conditions.

Hydrogen peroxide is a trigger of TNF-α production in co-culture with macrophages and intestinal epithelial cells stimulated with lentinan

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Lentinan has been known as an antitumor polysaccharide contained in *Lentinula edodes*, and its molecular weight was estimated to be more than 400,000. Lentinan shows the antitumor activity through host-mediated, because it does not kill cancer cells. However, it has not been ascertained whether or not lentinan was absorbed in intestine. In this study, co-culture system using intestinal epithelial cell line, caco2 cells (apical side), and macrophage cell lines, RAW 264.7 cells (basolateral side), was applied to clarify how lentinan stimulated macrophage. Caco2 cells (2.5×10^5 cells/well) were treated with 50μg/ml lentinan, and then were co-cultured with RAW 264.7 cells (5×10^5 cells/well). TNF-α production was detected just in basolateral side (150 pg), not in apical side. When catalase was added in basolateral side in the same condition, TNF-α production was inhibited to 50%, but superoxide dismutase did not. RAW 264.7 cells stimulated directly with lentinan did not produce TNF-α. Furthermore, when caco2 cells were cultured with lentinan at the same concentration without RAW 264.7 cells, they did not produce TNF-α in both sides. Neither lentinan was detected in basolateral side. These findings suggested that intestinal epithelial cells stimulated with lentinan produced H_2O_2 and newly produced H_2O_2 played an important role in cell to cell cross talking to enhance TNF-α production from macrophages.

Magnesium Deficiency Induces Accelerated Senescence in Human Fibroblasts

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Magnesium deficiency is surprisingly common in the United States; 10%-20% of the population consume less than half the RDA and many more are marginally deficient. Incidence of magnesium deficiency also increases with age. Interestingly, many disorders associated with magnesium deficiency are also common age-related conditions, such as cardiovascular disease, hypertension, and diabetes. Thus, inadequate magnesium make exacerbate age-related disease. These effects likely result from changes at the cellular level, as magnesium is an essential cofactor in over 300 enzymes and necessary for a vast number of cellular processes. Only a few studies on the cellular consequences of magnesium deficiency have been reported, but most have demonstrated a role for oxidative stress. Because oxidative stress plays a central role in aging and age-related disease, the effects of chronic magnesium deficiency in a human fibroblast model of cellular senescence were studied. Magnesium deficiency accelerated the senescent phenotype and increased staining for cellular senescence biomarkers in these cultures. Furthermore, cells in magnesium deficient conditions had greater sensitivity to oxidant challenge, reduced mitochondrial membrane potential, lower reduced/oxidized glutathione ratios, and higher levels of DNA-protein crosslinks. Given the importance of magnesium for proper cellular function yet the high incidence of dietary insufficiency, it is critical to define the long-term consequences of inadequate magnesium in human cells.

Do Antioxidants Trap Free Radicals *In Vivo*? The Critical Thinking Versus The Theory Of Reliability

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The free radical hypothesis of aging (Harman, 1954) has now been supported with a great deal of evidence. The random malfunctions of mitochondrial respiratory chains are of the first importance since they produce toxic superoxide radicals. Since all defense systems, among them – SOD and DNA-repair enzymes, operate with the limited reliability, free-radical damages accumulate up to the threshold dysfunction level. We estimated that the longevity of human brain could reach 250 years, should the reliability of the SOD-defense be perfect (Koltover, 1997). Furthermore, phenol antioxidants, among them – butylated hydroxytolene (BHT), when regularly introduced into diet, can increase the lifespan of animals (Harman, 1957; Emanuel, Lipchina, 1958). These chemicals indeed display the definite antioxidant properties in the model reactions of free radical peroxidation. However, BHT, along with ascorbic acid, vitamin E etc., is unable to compete with the specialized anti-oxidative enzymes for reactive oxygen species, considering that the rate constants and real concentrations of the "antioxidants" are too low in situ (Koltover, 1983; 2000). Having investigated the effects of BHT on the hormonal regulation and the low-temperature EPR signals in adult and old rats, we have revealed that BHT increases oxygenation of the heart tissue. Besides, BHT-induced EPR signal of the NO-hemoglobin complex arose in the animals' blood (Koltover, 1995). As hypoxia was shown to impair heart mitochondria and trigger superoxide release (Nohl, Koltover, Stolze, 1993), BHT and other phenol antioxidants, *in vivo* seems to perform the indirect anti-oxidative protection by means of fostering the supply of heart mitochondria with oxygen via the NO/hormonal mechanisms.

Modulation of acute pulmonary inflammatory response by myeloperoxidase

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Sepsis continues to elude effective therapy as mortality rates approach 50%, and thus remains an important human health problem without effective therapy. The lungs are generally the first to undergo failure, and this remains the most common cause of sepsis-induced mortality. While neutrophils play a key role in pulmonary failure during sepsis, the mechanisms remain incompletely characterized. Herein, we report that myeloperoxidase modulates acute pulmonary inflammatory responses induced by intranasal application of lipopolysaccharide (LPS). MPO-deficient mice had increased numbers of infiltrated neutrophil granulocytes in the bronchoalveolar lavage fluid (BALF) compared to wild-type mice at all time-points after LPS application. Additionally, other markers of lung injury and inflammation including total protein and IL-6 concentration in BALF were higher in MPO-deficient mice. Consistent with a more pro-inflammatory state, the level of the anti-inflammatory cytokine IL-12 was significantly lower in the BALF from MPO-deficient compared to wild-type mice after LPS application. Moreover, the degree of nitric oxide (NO) production (as measured by nitrite and nitrate in BALF) was significantly lower at early time points (8 and 24 h after LPS application) in MPO-deficient mice compared to wild-type mice. In contrast, NO production was significantly higher in MPO KO 72h after LPS application. These results suggest that neutrophil-derived MPO plays an important role in regulating the course of acute pulmonary inflammation, independent of its putative microbicidal functions, and may be linked to its ability to modulate the production and/or the bioavailability of NO.

Bioavailability and antioxidant activity of almond skin polyphenolics in hamsters and humans.

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The phytochemicals in nuts, found primarily in skins, may contribute to the observed inverse association between nut consumption and risk for heart disease. Therefore, we tested *in vitro* antioxidant activity of almond skin polyphenolics and investigated *in vivo* their bioavailability and antioxidant actions in hamsters and humans. Total polyphenolic content was 1.4 mg/g almond skins. *In vitro*, μM of polyphenolics increased the resistance of LDL oxidation as much as 7-fold in a dose-dependent manner and doubled the half-life of a-tocopherol. Polyphenolics interacted synergistically with added vitamin E to increase the lag time of LDL oxidation 30-100% over calculated additive effects. Hamsters were administered 8 mg polyphenolics (containing 594 μg of catechin, epicatechin, isorhamnetin, kaempferol, and quercetin) by gavage. These flavonoids were detected in plasma and liver after 1-5 h with their status in these tissues positively correlated with one another. Polyphenolics administration to hamsters enhanced LDL resistance to *ex vivo* oxidation. In contrast, in 7 healthy volunteers acute oral administration of 200 or 400 mg polyphenolics failed to have a direct effect on *ex vivo* LDL oxidation. However, the *in vitro* addition of vitamin E unmasked an effect of polyphenolics on LDL oxidation as evident by an increased resistance of LDL to oxidation compared to controls. Thus, almond skin polyphenolics enhance LDL resistance against oxidation in a synergistic manner with vitamin E, suggesting potential mechanisms of these phytochemicals independent of direct radical quenching.

Astaxanthin Rich Alga Meal Alleviate Reflux Symptoms in Non-Ulcer Dyspepsia Patients: A Randomised Double Blind Placebo Controlled Study

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Background: No standard therapy for non-ulcer dyspepsia (NUD) exists. In a pilot study with *H. pylori* positive dyspeptic patients astaxanthin rich algal meal effectively reduced intensity of functional heartburn. Aim: To evaluate the effectiveness of the carotenoid astaxanthin on dyspeptic symptoms in patients with NUD. Methods: Patients referred for upper endoscopy and with established NUD were included. They were randomly assigned to receive either 40 mg or 16 mg of astaxanthin in the form of algal meal of the green unicellular alga *Haematococcus pluvialis* (AstaCarox, AstaReal AB, Sweden) or placebo for 4 weeks. Dyspeptic symptoms were assessed with the gastrointestinal symptoms rating scale (GSRS); at entry, after 4-week treatment and 4 weeks after cessation of medicine. The primary objective of the study was GSRS measured after 4 weeks of treatment. Results: 131 NUD patients were included (mean age 43.9 ± 13.0). 43 patients received 16 mg of astaxanthin, 44 – 40 mg , 44 – placebo. Kruskal-Wallis test showed a statistically significant difference between two of the treatment groups for the variable Reflux syndrome. Pair-wise comparisions, regarding Reflux syndrome, were performed: Astaxanthin 16 mg vs. Astaxanthin 40 mg, p=0.0165; Astaxanthin 40 mg vs. Placebo, p=0.0337; Astaxanthin 16 mg vs. Placebo, p=0.8409. No other differences were observed. Conclusion: Study showed that 4-week treatment with 40 mg astaxanthin in form of algal meal reduce reflux symptoms in patients with non-ulcer dyspepsia significantly better compared to 16 mg or placebo.

Modulation Of Cutaneous Wound Healing by Ozone In Murine Model

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Ozone (O_3) is among the most reactive environmental oxidant pollutants to which cutaneous tissues are exposed. O_3 exposure has been shown to induce antioxidant depletion, oxidation of lipids and proteins within the outermost skin layer, the stratum corneum, as well as to induce stress/inflammatory responses in the deeper layers of the skin, epidermis and dermis. Wound healing is an essential process involved in regenerating tissue structure and function after being disrupted by a wide range of insults (physical, chemical, bacterial and viral). Cutaneous wound healing is a complex procedure associated with various factors including reactive oxygen species, nutrition and environmental factors such as O_3. To date, relatively little is known regarding the potential effects of O_3 on cutaneous wound healing. In the present study, hairless mice (4wks and 1 year old) full thickness excisional wounds were exposed to 0.8 ppm O_3 for 6 h. Rate of wound closure increased in old mice when compared to the air exposed mice while it was decreased in young mice. These results were accompanied by an increase in lipid peroxidation, as quantitated by an increases in 4-hydroxynonenal-protein adducts (4HNE) and a decrease in IkBa protein levels in the old mice when compared to the young mice. Collectively, our data demonstrate that O_3 is able to differently regulate cutaneous wound healing in both young and old mice. Further studies are needed to better elucidate the mechanism by which O_3 is able to modulate cutaneous wound healing.

Proteolysis in Cells Deficient in the 26S Proteasome S4 (ATP-ASE) Subunit

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The 26S proteasome is responsible for the degradation of most normal proteins in the cytosol and nucleus, whereas the 20S proteasome degrades most oxidatively damaged proteins. A polyubiquitin chain is used to target proteins to the 26S proteasome, and susceptibility to ubiquitinylation by a series of E3 enzymes largely determines the rate of turnover for many normal proteins. In contrast, the exposed hydrophobic residues of oxidatively modified proteins appear to be sufficient to target them to the 20S proteasome for degradation. The 26S proteasome can also degrade some oxidized proteins, but it does so rather inefficiently. We hypothesized that the S4 subunit of the 26S proteasome, a subunit with ATPase activity, might be required for ATP-dependent substrate unfolding by the 26S proteasome. We tested the involvement of ATP/ATPase activity, in human HB1080 connective tissue cells, with S4 subunit antisense and S4 subunit siRNA. Five days of treatment blocked more than 90% of the synthesis of the 26S proteasome S4 subunit. Surprisingly, a soluble extract from S4 depleted cells exhibited two- to three-fold higher degradation rates toward untreated (control) hemoglobin (Hb) than did normal cell extracts. In contrast, the S4 depleted extracts lost almost 50% of their ability to degrade oxidized Hb. These results may point to a key role for the 26S proteasome S4 subunit in the turnover of certain damaged proteins.

Intracellular Toxicity Of Alzheimer's Beta Amyloid Peptide: Mitochondrial Release of Oxidants and of Apoptogenic Signals

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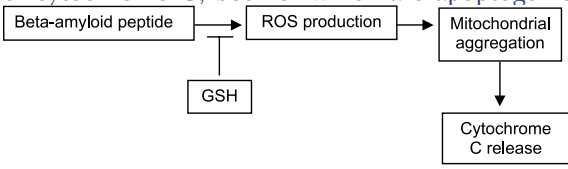
INTRODUCTION: Recent evidence suggests that intracellular accumulation of beta-amyloid peptide (Ab) plays an important role in the pathophysiology of Alzheimer's Disease. In particular, Takahashi *et al.* (2002) have found an accumulation of Ab in intraneuronal multivesicular bodies. Moreover, the implication of mitochondria in this process has been demonstrated (Morais-Cardoso *et. al* 2002).

AIM: We tested the hypothesis that Ab causes mitochondrial toxicity and that it increases mitochondrial free radical production and the release of apoptotic signals.

RESULTS: Ab peptide causes an increase in the rate of oxidant production by isolated mitochondria. Initially, these mitochondria were morphologically intact but after one hour of incubation with Ab they aggregated. Reduced glutathione (GSH) prevented this aggregation. After 6 hours of incubation, Ab peptide induced a released of cytochrome C from mitochondria.

DISCUSSION: Our results show that the production of free radicals by mitochondria is an early phenomenon in the toxicity of Ab peptide. Likewise, we found that it also caused mitochondrial aggregation and the released of cytochrome C, both of which are apoptogenic signals (Haga *et al.* 20



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REFERENCES

1. Takahashi RH, Milner TA, Li F, Nam EE, Edgar MA, Yamaguchi H, Beal MF, Greengard P, Gouras GK. (2002). Intraneuronal Alzheimer abeta-42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am. J. Pathol.* **161**: 1869-79.
2. Morais-Cardoso S, Swerdlow RH, Oliveira C (2002). Induction of cytochrome C-mediated apoptosis by amyloid b 25-35 requires functional mitochondria. *Brain Research* **931**: 117-125.
3. Haga N, Fujita N, Takashi T (2003). Mitochondrial aggregation precedes cytochrome C release

Effect of 3'-O-methylquercetin on adhesion molecule expression in human aortic endothelial cells

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Flavonoid intake has been associated with a decreased risk of cardiovascular diseases (CVD). Considering the role of inflammation in CVD, we studied the effect of 3'-O-methylquercetin (MeQ) on the expression of cellular adhesion molecules in human aortic endothelial cells (HAEC). We found that MeQ inhibits TNF-α-induced expression of intercellular adhesion molecule-1 (ICAM-1) and E-selectin (ES) in HAEC. Here we studied whether this effect of MeQ is due to modulation of intracellular signaling or extracellular binding and inactivation of TNF-α. HAEC were incubated (17.5 h) with inhibitors of nitric oxide (L-NAME, 0.5 mM) or GSH synthesis (BSO, 1 mM), in the absence or presence of MeQ (10 μM), and subsequently co-exposed to TNF-α (100 U/ml, 7.5 h). Neither L-NAME nor BSO affected the inhibitory effect of MeQ on adhesion molecule expression. To evaluate a possible interaction between TNF-α and MeQ, HAEC were incubated with MeQ, washed and then incubated with TNF-α, and MeQ was added at different intervals thereafter. Results showed that the inhibitory effect of MeQ on adhesion molecule expression was similar at all time points. When experiments were repeated without pre-incubation with MeQ, but MeQ was added together with TNF-α, the inhibitory effect of MeQ on ES was abolished. In summary, neither nitric oxide or GSH, nor binding to TNF-α seems to mediate the attenuation of adhesion molecule expression by MeQ. More work is required to elucidate the mechanism(s) by which MeQ inhibits adhesion molecule expression in HAEC.

Recycling of ascorbic acid in erythrocytes of smokers and nonsmokers

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Recycling of ascorbic acid (AA) is vital to the preservation of its antioxidant function and the AA pool. In particular in smokers, who are subject to increase oxidative stress from the inhalation of gas phase and other radicals, this process must be of major importance. We have compared the recycling of AA in freshly isolated erythrocytes of heavy smokers to that of nonsmokers in two separate studies with a total of 20 and 80 subjects, respectively. The results show that AA recycling rate is significantly higher in smokers than in nonsmokers ($p<0.0001$). This effect was independent of AA transport, energy status, intracellular glutathione concentration, plasma AA concentration, and four weeks of AA supplementation. However, a significant positive correlation with plasma malondialdehyde was found ($p<0.01$). In conclusion, our results suggest that AA recycling activity is increased by smoking—perhaps as a preventive measure—and that dehydroascorbic acid reductase activity may be induced by oxidative stress.

The Interference of Rosmarinic Acid in the DNA Fragmentation Induced by Osmotic Shock

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The aim of our study was to investigate both the pro-oxidant and antioxidant activity of rosmarinic acid and curcumin and the ability of rosmarinic acid to block apoptosis, using sorbitol as inducer of cell death by ROS in K562 cells. We examined K562 cells treated with rosmarinic acid and curcumin for DNA fragmentation: the cells treated with 10, 15 and 25 μM rosmarinic acid and 10, 15 μM curcumin did not show fragmented chromosomal DNA, while the cells treated with curcumin at 25 μM showed fragmented chromosomal DNA. We assayed for the ROS content in order to confirm the involvement of oxidative stress in sorbitol-mediated cell death. Cells were loaded with the peroxide-and hydroxyl-radical sensitive fluorophore DCF-DA and assayed by FACS analysis. K562 cells treated with 1 M sorbitol had markedly higher level of DCF fluorescence than cells treated with both rosmarinic acid and sorbitol. Furthermore, we found that the caspase-9, in K562 cells treated with 1 M sorbitol, showed a higher protein level respect to cells treated with both compounds. In conclusion, the antioxidant activity of rosmarinic acid is able to inhibit sorbitol-induced apoptosis

Evaluation of GeneChips to Define Sensitivity and Specificity of Tissue Specific mRNA Expressions

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Analysis of genome wide changes in the expression of mRNAs and proteins, "global functional genomics", provides voluminous molecular data for mining the potential roles of mRNAs and proteins in determining physiological and pathological states. We have evaluated the utility of high density oligonucletide arrays, GeneChips, to identify tissue specific mRNA profiles and correlated it with well defined biochemical properties of the tissues from C57Bl6, normal and transgenic mice. Total RNA was isolated from each tissue (lung, liver, heart, brain and adrenal glands) and processed to obtain biotinylated RNA using the manufacturers's (Affymetrix) protocol. Mu74Av2 and M430A arrays were used to obtain mRNA expressions. 2000-5000 mRNA transcripts were reliably ($p < 0.05$) detected. The detection signal ranged from 10-6000 units, probably reflecting the abundance of the respective mRNAs. Transcripts for hemoglobin alpha and beta chains were detected at equivalent signal intensities and reflect the stoichiometric amounts of the encoded proteins. Tissue specific mRNAs could be identified which also correlated well with the published reports of relative abundance of the encoded protein. Similarly mRNAs encoding enzymes of the intermediary metabolism were detected and correlated well with the tissue specific expression of the enzyme activities. The expressions of mRNAs for catalases, superoxide dismutases, glutathione peroxidases and cytochrome P 450s were detected in all the tissues and showed tissue specific expressions. These comparative analyses suggest that GeneChip assays provide reliable estimates of relative mRNA expressions that are tissue specific and may be reliably used to define tissue specific changes in mRNAs in response to biological stimuli.

Human Breastmilk Storage And Oxidative Stress

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Breastfeeding and human milk are recognized as optimal for human infants. Among the situations that oblige to abandon human lactation is found the maternal need to be stayed away for its early incorporation to the labor life. This situation requires new resources that make possible to prolong maternal lactation in these circumstances. Human breastmilk storage for use later is on the increase as a result of economic activities of mothers. It is thought that free radicals play a role in the pathogenesis of several disease processes in premature infants. The aim of this study was to assess the changes in an lipid peroxidaion indicator (MDA) and in glutathione peroxidase (GPx) activity when human milk was kept in refrigeration during 48h. or in congelation during 10 days.

RESEARCH DESIGN AND METHODS:

25 human milk samples are analyzed. Samples were divided in three parts, first to be analyzed inmediately, second is left in refrigerator 4° C and it is analyzed 24h after and third is frozen at –20° C and it is analyzed 10 days after. MDA concentration was measured by liquid cromatography according to the method of Richard et al.(92) GPx activity was measured according to the method of Lawrence et al (78).

RESULTS:

MDA was significantly increased after 10 days at –20° C . There was no difference between MDA from fresh milk or refrigerated milk. GPx activity was significantly increased in refrigerated milk and in frozen milk. We conclude that it seems better to freeze milk than to refrigerate it in order to prevent oxidative stress in milk samples.

Superoxide Dismutase Does Not Govern Longevity in *Drosophila*

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The relationship between superoxide dismutase (SOD) activity and the life span of the fruit fly, *Drosophila melanogaster*, is a subject of ongoing controversy. Overexpression of this antioxidative enzyme would be hypothesized to minimize free radical attacks and the resulting molecular oxidative damage; consequently, increasing SOD activity should decrease the rate of aging and extend the life span of the fly. However, flies overexpressing either genomic Cu-Zn SOD or Mn SOD, or both enzymes together with increased catalase, failed to exhibit any extension of life span (*J. Biol. Chem.* 278: 26418-26422). In a study of human wild type and FALS-associated mutant Cu-Zn SOD enzymes in a *Drosophila* Cu-Zn $SOD^{-/-}$ background, decreasing Cu-Zn SOD activity by 90-95% did not typically cause any decrease in life span (*Proc. Natl. Acad. Sci. USA* 100: 301-306). Conversely, flies genetically selected for long and short life spans exhibited no significant differences in total or Mn SOD activities. Reports by some investigators have purportedly revealed conditions of overexpression or specific tissues in which SOD activity levels do govern the rate of aging of the flies. However, the predicted correlation between SOD activity and life span occurs only when the life span of the control flies is abnormally short. In a majority of studies, no consistent relationship is observed (reviewed in *Free Radic. Biol. Med.* 33: 575-586). Thus, the existence of a causal connection between the level of SOD expression and aging in *Drosophila* is not supported by currently-available evidence.

Superoxide Dismutase Mimetics Do Not Increase Growth of Saccharomyces Cerevisiae Mutants Lacking Cu/Zn Superoxide Dismutase

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Saccharomyces cerevisiae mutants lacking cytosolic Cu/Zn superoxide dismutase (sod1-) exhibit a wide variety of phenotypes related to the mutant's increased levels of oxidative stress such as poor aerobic growth and air-dependant auxotrophies for methionine and lysine. With the current interest in synthetic SOD mimetics, we have attempted to rescue the sod1- yeast using a variety of mimetics. So far, our results indicate that compounds from Eukarion and Metaphore, as well as manganese tetrakis (4-benzoic acid) porphyrin (MnTBAP), a Mn-porphyrin SOD mimetic, are unable to aid our yeast mutants and are in some cases actually toxic. It is not clear why yeast are not rescued with these compounds as SOD mimetics rescue a variety of phenotypes in other model systems.

The Role of the Lon Protease in Maintaining Mitochondrial Homeostasis

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The Lon protease is a multifaceted protein. Studies from an array of organisms reveal that Lon participates in mitochondrial homeostasis by regulating gene expression, maintaining the genome, biogenesis, and possessing a chaperone-like function in the matrix. Which functions are conserved in humans, and how these roles relate to one another is not well understood. Previously, we have shown that the downregulation of Lon in human fibroblasts results in increased levels of oxidized proteins, such as aconitase (a marker for aging). The downregulation of Lon also leads to impaired mitochondrial function and increases cell death, partly due to the loss of Lon's chaperone-like function. Here we show that the human Lon protease is also involved in mitochondrial biogenesis, where the decrease in Lon protein is associated with a decrease in mitochondrial mass. We also show that Lon is regulated by oxidative load, in which an increase in oxidized protein results in a rapid increase in Lon expression. Thus, Lon not only reduces oxidative load but is also stimulated by oxidative stress. We propose that the Lon protease may serve at least a dual function in maintaining mitochondrial homeostasis in humans. Firstly, Lon might protect against mitochondrial protein damage from free radicals during oxidative stress by proteolysis and chaperone-like functions. When stress conditions have subsided, Lon may be regulating mitochondrial biogenesis by acting as a replication or transcriptional regulator. Thus, defects in Lon regulation can lead to aging and disease.

Estradiol Regulation of Brain Mitochondrial Function

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Estradiol (E2) is neuroprotective against neurodegenerative insults that are associated with calcium dysregulation and increased oxidative damage. These neuroprotective effects of E2 are dependent upon intact mitochondrial function. Using Fura4F and RhodFF to simultaneously monitor cytosolic ([Ca2+]i) and mitochondrial ([Ca^{2+}]m), respectively, we showed that E2 pretreatment of hippocampal neurons attenuated the rise in [Ca^{2+}]i while significantly potentiating the rise in [Ca^{2+}]m in response to excitotoxic glutamate. Although enhanced Ca^{2+}levels can be beneficial for cellular signaling, excessively high [Ca^{2+}]m leads to apoptosis and presumably potentiation of the rise in [Ca^{2+}]m would exacerbate toxicity. E2 treatment of ovariectomized rats attenuates the Ca^{2+}-induced decline in respiratory activity of isolated brain mitochondria. Apoptosis during neurodegeneration is preceded by release of cytochrome c from the mitochondrial intermembrane space. This translocation is regulated by the expression and localization of the Bcl-2 family of proteins. We showed that E2 prevents the glutamate- and βA-induced reciprocal mitochondrial/ cytosolic translocation of cytochrome c and Bax. These data suggest a mechanism of E2 neuroprotection involving an increase in Ca^{2+} sequestration by mitochondria coupled with an increase in mitochondrial Ca^{2+} load tolerability and an increase in mitochondrial respiratory function.

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Antioxidant Activities of Novel α-Lipoic Acid Derivatives: N-(6, 8-Dimercaptooctanoyl)-2-Aminoethanesulfonate- and N- (6, 8-Dimercaptooctanoyl)-L-Aspartate-Zinc Complex

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Two new compounds, sodium N-(6, 8-dimercaptooctanoyl)-2-aminoethanesulfonate- and sodium N-(6, 8-dimercaptooctanoyl)-L-aspartate-zinc complex were synthesized from α-lipoyl-2-aminoethanesulfonate and α-lipoyl-L-aspartate by reduction of zinc/acetic acid respectively. These α-lipoyl-amino acids were obtained by a coupling of α-lipoic acid and 2-aminoethanesulfonate or L-aspartate, using a mixed anhydride method. Scavenging activities of these derivatives against hydroxyl radicals (•OH) was demonstrated directly using electron spin resonance (ESR) spectrometry with spin trapping. Otherwise an apparent superoxide anion radical ($O_2^{\bullet-}$) scavenging effect of these derivatives may be due to the inhibition of $O_2^{\bullet-}$ generation system, i.e., xanthine oxidase. Scavenging activities of these compounds against nitric oxide radicals (NO•), and peroxynitrite (ONOO-) were estimated by the flow injection analysis using the Griess reagent and by a fluorescence spectrometry using dihydrorhodamine 123, respectively. Meanwhile, these derivatives showed protective effects against lipid peroxidation and carbonyl protein formation. Scavenging activities against NO• and ONOO-, and inhibitory effects on protein carbonyl formation of these derivatives were much stronger than these of α-lipoic acid itself.

Catalysis of UV-Induced DNA Damage: Comparison of Folic-Acid with 5-Methyltetrahydrofolate

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UVA radiation has been connected with skin aging and carcinogenesis. Most of its cellular effects are mediated by singlet-oxygen photochemical-generation after excitation of cellular photosensitizers. Exogenous photosensitizers from the diet are also considered to participate in oxidative reactions leading to solar carcinogenesis. Folate, an essential B vitamin is predominantly present in the human body and in foods in reduced forms, mostly 5,6,7,8-tetrahydrofolate and its one-carbon derivatives. The synthetic folic acid (FA) used for food fortification and in supplements is an oxidized form not present in significant quantities in fresh foods. Due to the possible combination of US grain-fortification program, cereal consumption and multivitamin supplementation, many people might be exposed to greater than 1 mg/day of FA. FA is known to undergo photodegradation and may generate photosensitizing by-products in human skin. Singlet oxygen has been previously suggested to participate in this mechanism. Our results show that FA is decomposed within 40 min under exposure to UVA. The initial photoproducts were 6-formyl-pterin (6fp) and PABA-glutamic acid. In this reaction, FA disappeared quickly, as well as a major part of 6fp, to give an increasing amount of pterin-6-carboxylic acid (PCA). The reduced form, 5-methyltetrahydrofolic acid (5MTHF) exposed to UVA did not degrade more than would be expected from its autooxidation in the absence of light. In the presence of FA, PCA or 6fp supercoiled-plasmid DNA was degraded during exposure to UVA. Thus, FA but not 5MTHF, the natural form in plasma, catalyses UV-induced DNA damage. The possibility of FA exacerbation of skin cancer must be taken into account when consuming supplements and fortified foods.

A Flow-Cytometry Based Micronucleus Assay

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Exposure to stresses such as smoking, radiation or deficiency of the vitamins folate, B6 or B12 can cause hazardous lesions in DNA including double-strand breaks. Double-strand breaks result in small fragments of chromosome, which form a secondary nucleus (micronucleus) outside the main nucleus of a dividing cell. In red blood cells (RBCs), the micronucleus is left behind after the nucleus is extruded to form the mature reticulocyte during erythropoiesis. Micronucleated reticulocytes in the human peripheral blood are not generally available for analysis because the spleen quickly removes aberrant RBCs. We have developed a method to isolate and analyze young reticulocytes in the peripheral blood for the presence of micronuclei. This method applies flow-cytometry to measure micronuclei in an enriched transferrin-positive young reticulocyte population. The effect of tobacco smoking on chromosome damage has been the object of many studies. The measurement of micronuclei in peripheral blood lymphocytes has been accepted as the most suitable biomarker in assessing oncogenic risk. The traditional cytokinesis-block micronucleus (CBMN) assay applies chemicals to block cytokinesis and the micronuclei are manually counted and scored using microscopy. In the present study, we have used a novel flow-cytometry based micronuclei-assay to assess DNA damage in smokers. Heavy smoking is associated with increased micronuclei frequency. The results show that this assay can effectively detect micronuclei in human blood samples. This assay is simple and unlike the traditional CBMN assay, allows rapid evaluation of a large number of cells and therefore will prove to be an excellent tool for genetic monitoring of human populations.

Inactivation of NADP$^+$-dependent isocitrate dehydrogenase by glycation *in vitro* and *in vivo*

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Recently, we demonstrated that the control of cytosolic and mitochondrial redox balance and the cellular defense against oxidative damage is one of the primary functions of NADP$^+$-dependent isocitrate dehydrogenase (ICDH) through to supply NADPH for antioxidant systems. When exposed to reducing sugars such as glucose, glucose 6-phosphate dehydrogenase, and fructose, ICDH was susceptible to oxidative modification and damage, which was indicated by the loss of activity, fragmentation of the peptide as well as by the formation of carbonyl groups. The glycated ICDH was isolated and identified by boronate affinity chromatography and immunoblotting with anti-hexitollysine antibody. Glycation of ICDH was protected by antioxidant enzymes, free radical scavengers, and spin-trapping agents. The structural alterations of modified enzymes were indicated by changes in thermal liability, intrinsic tryptophan fluorescence, and binding of the hydrophobic probe 8-anilino-1-naphthalene sulfonic acid (ANSA). Levels of glyated ICDH, which was purified by immunoprecipitation and probed with anti-hexitollysine antibody, were increased in the kidney of streptozotocin-treated rats and (lens from diabetic patient also suffered to cataract). The decrease in ICDH activity and the modulation of cellular redox status was observed from those tissues. We also found that levels of glycated ICDH increased in IMR90 cells and rat kidney during normal aging. The glycation-mediated damage to ICDH may result in the perturbation of cellular antioxidant defense mechanisms and subsequently lead to a pro-oxidant condition and may controbute to various pathologies associated with the general aging process and long term complications of diabetes.

Lens Mitochondria, ROS and Cataract

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The lens of the eye represents an organ with a single epithelial cell type that differentiates into all of the functional cells therein. As such, it provides an organelle, isolated from direct blood supply, that undergoes a gradually developing ROS-related pathology in all mammals and birds i.e., cataract. We find that both the surface nucleated lens epithelial cells (LEC) and their descendant interior anucleate, amitochondriate lens fibers develop deteriortive changes with age in normal mice and reflect the presence or absence of anti-oxidant enzymes in mutant mice. Using fluorescent dyes and dual laser confocal assessment, we measured: 1) LEC mitochondrial membrane potential (MMP) by CMX rosamine, with protein mass control by Mitotracker green uptake, 2) lens peroxide production by dichlorofluorosine (DCF); 3) O_2 utilization by reduced rosamine; 4) DNA retention in lens fibers by Hoechst dye.

Results: 1) Lens opacities progressed with age in both normal and the below-noted mutant mice, 2) In all old mice there was reduced LEC utilization of O2, as well as increased lens peroxide content; 3) In all old mice there was retention of DNA in and around nuclear and mitochondrial remnants in deeply internalized fiber cells, and this was localized at the sites of peroxide presence and lens opacity; 4) lenses of old glutathione peroxidase-1 homozygous knockout mice (GPX-1 KO) had more severe cataracts and greater reduction of O_2 utilization than same age controls; 5) in long-lived dwarf (GHRKO) mice with non-functional growth hormone receptors and almost no circulating IGF-1 there was a delay in age-related cataract formation coupled with a reduced MMP in the young mutants versus normals. Our findings confirm the importance of antioxidant protection against cataract and provide a mechanism for ROS-driven, and DNA fragment retention-driven development of age-related cataract.

α-Tubulin Detyrosination: A Redox-Sensitive Regulatory Switch for Vascular Smooth Muscle Cell Growth

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Hyperproliferation of smooth muscle cells is a hallmark of vascular diseases such as atherosclerosis and is a prominent complication arising from restenosis following angioplasty. Whereas recent studies suggest that oxidants play a role in the hyperproliferative and hypertrophic responses of smooth muscle cells (SMCs), the mechanisms remain poorly characterized. Herein, we provide evidence that H_2O_2 produced by the nonphagocytic NAD(P)H oxidase Nox1 is an important redox mediator of SMC proliferation, and that this may be directly linked to posttranslational modifications of a-tubulin. While serum-starved rat aortic SMCs are essentially devoid of Nox1 expression, treatment with PDGF or AngII upregulates the expression of this NAD(P)H oxidase. In parallel, the levels of detyrosinated a-tubulin are markedly increased and temporally follow the cell cycle. Therefore, upregulated detyrosination of a-tubulin is a common feature of SMC proliferation and hypertrophy, potentially linked to the induced expression of Nox1 and H_2O_2 production. Supporting this notion, NIH 3T3 fibroblasts stably transfected with Nox1 reveal increased levels of detyrosinated a-tubulin, and co-expression of catalase reverses this effect. It was also found that 3-nitrotyrosine, which is irreversibly incorporated into the C-terminus of α-tubulin, blocked the proliferative response of Nox1 transfected cells, suggesting that α-tubulin detyrosination state may be an important factor in the proliferative response. Collectively, our data suggest that Nox1 expression is upregulated in PDGF- and AngII-activated smooth muscle cells, and that H_2O_2 derived from Nox1 induces characteristic posttranslational modifications to a-tubulin.

DETOX, a New Combination of Topical Anti-Oxidants, Protects Skin from Oxidative Stress

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Environmental and oxidative stressors continuously challenge the skin, resulting in accelerated aging. In response to these aggressors, skin cells employ a variety of antioxidant defenses. The supply of such cutaneous antioxidants decreases with age and with an increased exposure to environmental stressors. Consequently, topical formulations containing natural antioxidants could replenish cellular defenses. A new formulation, DETOX, contains a combination of active natural antioxidants and an ultra-violet light filter. The present study was conducted to determine the protective properties of DETOX formulations against external aggressors. The antioxidant activity of DETOX was verified against the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) with an IC50 of 5.95 mg/ml. Topically applied DETOX reduced the loss of cell viability (91.8%) and the increase in the pro-inflammatory mediators IL-1α (92.8%), IL-8 (84.1%) and the release of active matrix metalloproteinase-1 (15.6%) induced by solar-simulated UV light in a human epidermal equivalents tissue model. Topically applied DETOX minus the ultra-violet light filter also significantly reduced the loss of cell viability (71.0%) and the release of IL-1α (72.7%) and IL-8 (58.8%). Taken together, the DETOX formulation delivers photo-protective, and antioxidant properties that can help to replenish epidermal anti-oxidants and protect against aggression-induced aging.

Risk for cataract and atherosclerosis due to alcoholic beverage consumption is related to plasma antioxidant or pro-oxidant activity

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Objectives: To correlate the oxidative state of post-absorptive blood plasma after consumption of one or three drinks of different beverages with known J-shaped epidemiological risk curves. Design: plasma antioxidant or pro-oxidant activity in human volunteers was measured after one or three typical drinks containing equivalent amounts of alcohol (except for an alcohol-free stout).

Results: One drink of red wine, lager beer, or stout(5% alcohol v/v, and alcohol-free) increased the antioxidant activity in plasma samples. Three drinks of red wine, lager beer, or stout(5% alcohol v/v, and alcohol-free) increased pro-oxidant activity in plasma samples. For a solution of alcohol three drinks resulted in pro-oxidant plasma, while one drink did not increase plasma antioxidant activity . Two volunteers, who took longer to metabolize ethanol after ingestion of lager beer and red wine, had elevated antioxidant activity in plasma. Conclusions: One drink of red wine, beer, or stout provided equivalent increases in plasma antioxidant activity. Three drinks of red wine, beer, or stout provided equivalent increases in plasma pro-oxidant activity. This may partly explain the decreased risk of cataract and atherosclerosis from daily consumption of one drink as well as the increased risk from daily consumption of three drinks of alcoholic beverages. The plasma pro-oxidant activity appears to be due to ethanol metabolism, while the antioxidant activity may be due to the absorption of polyphenols in the beverages. Stout increased LDL oxidation lag time. Stout itself was shown to destroy superoxide.

Acute Ethanol-induced Alterations on the Rat Central and Peripheral Nervous Tissue

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Ethanol metabolism is able to generate oxidative stress in different tissues. The effects on the central and peripheral nervous system of the rat, of a single hypnotic sub-lethal dose (3 g/kg, i.p.) of ethanol have been investigated. As a control, the nonmetabolized alcohol tertiary butanol was administered at a dose previously reported to exert the same physico-chemical effects at the cellular level (0.6 g/kg, i.p.). Acute ethanol administration induces glutathione depletion, decrease in glutathione peroxidase activity, and increase of malondialdehyde concentration in rat sciatic nerve, 60 min after ethanol administration. These changes did not appear in tertiary butanol-treated animals, and could not be established in hippocampal tissue of any group. Moreover, ethanol administration induced a complete blockade of extracellular-recorded muscle action potentials on the dorsal musculature of the rat's tail after 59 ± 5 min (n = 8). Prior to this blockade, there was a significant decrease in the motor nerve conduction velocity of the faster caudal nerve fibers. The peripheral nervous system dysfunction observed, only appeared in ethanol-treated rats but not in tertiary butanol-treated animals, whereas both alcohols elicited similar systemic effects. Electron microscopy analysis showed relevant axonal myelin degeneration of these fibers only in sciatic nerves from ethanol-treated animals. These data confirm our previous hypothesis that peripheral nervous tissue is more susceptible to ethanol oxidative toxicity than the central, and that this toxicity depends on ethanol metabolism. Supported by grants PRUCH/03-06, from the Dirección General Drogodependencias (Generalitat Valenciana) and Plan Nacional contra la Droga (Minsterio del Interior).

Peroxiredoxin function is controlled by p53-modulated sestrins, homologs of bacterial AhpD

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Hydrogen peroxide serves as a secondary messenger in numerous signaling pathways. To transduce its signal, hydrogen peroxide shuts off the normal antioxidant defense by transiently overoxidising peroxiredoxins (Prxs), the major thiol-containing enzymes that control peroxide levels. After the signal has been conveyed, the antioxidant firewall needs to be reestablished to prevent oxidative stress. Though, the factors responsible for reactivation of overoxidised Prxs containing Cys-SO_2H remain unknown. We show that this reaction are mediated by the p53-regulated sestrin-family proteins PA269 and Hi95 that contain predicted redox-active domain homologous to prokaryotic AhpD, an enzyme catalysing reduction of bacterial peroxiredoxin AhpC. We demonstrate that sestrins bind to Prxs and modulate retroreduction of their overoxidised forms *in vivo* and *in vitro*. Substitution of the predicted redox-active cysteine or inhibition of sestrins by siRNA abolishes this effect leading to an increase in peroxide levels and oxidative stress. The results suggest that unlike bacterial AhpD, which is disulfide reductase, eukaryotic sestrin have been evolved to support previously unknown type of enzymatic reaction in mammals. Since sestrins are modulators of peroxide signaling and antioxidant defense, they might be excellent targets for novel drugs against ROS-related pathologies and aging.

LPS induced lung inflammation in alpha-tocopherol transfer protein null mice

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Oxidative stress accompanies many lung diseases and may contribute to their pathogenesis. Alpha-tocopherol (αT), the dominant lipid soluble antioxidant in humans, may be critical for protection against oxidative injury. Recent studies suggest that αT has non-antioxidant function and may modulate immune processes, but the function of αT in the lung is not understood. To study the role of αT in lung inflammation, we used mice with a deletion in the αT-transfer protein gene (TTP-/-) with lung αT levels 1/10 of control (WT). Mice (35IU αT/kg diet) were sacrificed (n=3) 2, 4, 8, 24 and 48h after intranasal LPS (0.6 g/g) or saline exposures. Blood was withdrawn, lungs lavaged (PBS, 2x1ml) and collected. Plasma and lavage supernatants were assayed for aT (HPLC), TNF, MIP-2 & sICAM (ELISA). Cell counts and lavage NOx were analyzed to assure lung inflammation. Within each genotype, lung αT levels were unchanged during the course of inflammation. Lavage NOx & sICAM concentrations peaked 2h after LPS and were similar in TTP-/- and WT mice. Highest TNF concentrations were found 2h after LPS exposure, and were significantly higher in TTP-/- mice (mean±SE: 6.1±0.3 vs. 15.9±1.7 pg/ml, p=0.03). 4h after LPS, lavage fluid from TTP-/- mice showed higher MIP-2 levels (1.5±0.01 vs. 25.1±0.06 pg/ml, p=0.01) and more neutrophils (19.0±3.1 vs. 49.5±4.0 104/ml, p=0.03). Plasma TNF and sICAM concentrations were not different in TTP-/- and WT mice. Our data suggest that relative αT deficiency may increase the inflammatory response in the lung.

Anti-oxidized low-density lipoprotein autoantibodies in human coronary atherosclerosis

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Recent data including laboratory data, animal models and human studies support an association between anti-oxidized low-density lipoprotein (oxLDL) and atherosclerosis. Usually in human studies, anti-oxLDL levels are elevated in patients with more advanced atherosclerosis as demonstrated by carotid ultrasound studied and coronary angiography. However, animal models of immunization with oxLDL resulted in protection from atherosclerosis in the presence of high levels of anti-oxLDL antibodies. I have demonstrated elevated levels of anti-oxLDL autoantibodies in patients having coronary artery disease compared with controls. Other anti-phosholipid antibodies (such as anti-cardiolipin) levels were also elevated in patients with significant coronary artery stenosis as demonstrated by coronary angiography compared with patients without significant stenosis (0.27 ± 0.15 versus 0.17 ± 0.8; $P < 0.01$). The status of risk factors for coronary artery disease has been evaluated in the coronary artery disease patients. Anti-oxLDL antibody levels were unaffected by the presence or absence of these risk factors. There was no correlation between coronary calcification as determined by spiral CT and levels of any of the autoantibodies tested. Moreover, anti-oxLDL levels were elevated in patients without any coronary calcification compared with patients with some degree of coronary calcification (0.19 ± 0.18 versus 0.12 ± 0.13; $P < 0.05$). These results support an association between autoantibodies and presence of coronary artery disease, but the nature of association with atherosclerosis is more complicated. It is possible that anti-oxLDL autoantibodies contribute to manifestations of coronary artery disease rather than associated with total amount of atherosclerosis.

Non-Invasive Raman Spectroscopy Measurement of Human Carotenoid Status

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Carotenoids are an important group of dietary antioxidants and are believed to be responsible for many health benefits. Serum or plasma carotenoid measurements are commonly used to assess human carotenoid status and to monitor reported intake of fruits, vegetables and dietary supplements in epidemiological research. Recently, a Raman-spectroscopic method was developed to safely assess skin carotenoids non-invasively (Biophotonic Scanner). To help validate this method, 104 healthy adults (64 men, 40 women) were recruited for this study. After an overnight fast, each subject provided a blood sample, and skin carotenoids were assessed at the palm of the hand using Raman spectroscopy (RS) with an excitation wavelength of 473 nm. Blood was processed for serum, which was analyzed for carotenoids by HPLC. Results show a highly significant correlation between serum total carotenoids and skin carotenoids as assessed with RS ($r = 0.78$, $p < 0.001$). Mean serum total carotenoid concentration was 1.44 mcg/ml (range: 0.37 – 3.36) and the mean Raman response for skin measurements was 28,808 counts (range: 14,524 – 56,298). Among individual carotenoids, correlations were strongest for beta-carotene, followed by alpha-carotene, lutein/zeaxanthin, lycopene and beta-cryptoxanthin. Based on these results, RS is able to estimate serum total carotenoids with a variability of +/- 10 % and 95 % confidence. This high correlation between serum and skin carotenoid measurements helps validate RS as a novel, non-invasive, rapid, and field-usable tool to assess human carotenoid status.

Redox Regulation of α-Tubulin Posttranslational Modifications in Prostate Cancer

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Prostate cancer is the most frequently diagnosed malignancy and the second leading cause of cancer- related death in men. Whereas recent studies suggest that oxidants play a role in prostate cancer, the mechanisms remain poorly characterized. Herein, we provide evidence that hydrogen peroxide (H_2O_2) produced by the nonphagocytic NAD(P)H oxidase Nox1 is an important redox mediator of prostate cancer, and that this may be directly connected to posttranslational modifications of α-tubulin. While normal prostate epithelial cells (PrEC) are devoid of Nox1 expression, androgen-dependent prostate cancer cells (LNCaP) express this NAD(P)H oxidase. In parallel, the levels of detyrosinated α-tubulin are nearly absent in PrEC, but highly expressed in LNCaP cells. Additionally, acetylated α-tubulin is highly expressed in Prec, but absent in LNCaP cells. Therefore, upregulated deacetylation and detyrosination of α-tubulin is a common feature of cultured prostate cancer cells potentially linked to exuberant expression of Nox1 and H_2O_2 production. Collectively, our data suggest that Nox1 expression is upregulated in prostate cancer cells, and that H_2O_2 derived from Nox1 induces characteristic posttranslational modifications to α-tubulin.

Degradation of Oxidized Proteins by the Immunoproteasome

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The immunoproteasome has been found to be involved in intracellular antigen processing for MHC class I antigen presentation. Little is known about the substrates preferred by the immunoproteasome. Since the structure of the immunoproteasome is very similar to the 20S core proteasome, we can infer that these two proteasomal forms might degrade similar substrates. Our laboratory has previously shown that the 20S core proteasome preferentially degrades oxidized proteins without the aid of ATP or ubiquitin conjugation. We hypothesized that the immunoproteasome would also be able to degrade oxidized proteins in preference to native proteins, and that the products generated would be good substrates for MHC class I antigen presentation. Consistent with this proposal, antisense oligonucleotides to both the C2 and C5 proteasome subunits effectively blocked production of MHC class I molecules on the cell surface of Jurkat T cells. We are now conducting in vitro studies to characterize the protein degradation activity of the immunoproteasome. Our results show that oxidized proteins are the preferential substrates of the immunoproteasome. Since all cellular proteins are subject to oxidation, our results may provide a simple and universal mechanism by which cells are able to present self-antigens for self-recognition by the immune system, and intracellular foreign antigens (i.e. viral proteins) for the destruction of the infected cell.

Cigarette Smoke-Induced Stress Responses in Murine Skin

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Exposure to environmental tobacco smoke (ETS) has shown a negative impact on public health worldwide. ETS contains at least 250 toxic or carcinogenic chemicals, which has been known as highly reactive components causing inflammation, nitration and adduct formation as well as oxidative modification of lipids, DNAs and proteins. The effects of ETS on internal organs including lung, bladder and cervix have been studied extensively. Furthermore, epidemiological studies have indicated an association between TS and skin aging. Therefore, ETS may have a high impact on skin disease morbidity. However, the exact mechanism of ETS-induced premature skin aging is still unknown. In this study, hairless mice were exposed to ETS for 5 days (6 hr per day) at concentration of 60 mg/m3 particles. ETS showed increased stress proteins, HSP27 and heme oxygenase-1, starting at 1 day after ETS exposure. After 5 days of ETS exposure MMP-9 activity and its mRNA level were increased compared to the air exposed mice. These data suggest that injurious-reparative processes are activated by ETS exposure. In many skin inflammatory disorders, MMP-9 expression has been associated with the degradation of the basal membrane and with a migratory regenerative status of keratinocytes. In this regard, ETS-induced MMP-9 in skin tissues may contribute to enhance skin diseases and not last ageing and wrinkle formation. Collectively, our data demonstrate that exposure to ETS is detrimental in skin biology.

Transcription factor NF-κB as a potential biomarker of oxidative stress to evaluate antioxidant effects in humans

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There is increasing interest in the involvement of transcription factors, such as of the transcription factor NF-κB (nuclear factor-κB), in the pathogenesis of various diseases. The involvement of NF-κB is especially of interest as it is activated by oxidative stress and its activation can be modulated by antioxidant compounds. The activation of NF-κB can be determined by the electromobility shift assay (EMSA) or by an ELISA-based assay with NF-κB bindingsite-specific probes. We performed several studies using human mononuclear cells isolated from peripheral blood, showing that the assay is applicable for clinical trials [1,2,3,4]. In current study we performed a randomized crossover human volunteer trial in which we performed NF-κB assays in combination with other risk factors linked to oxidative stress, such as F2-isoprostanes, and show that this combination strengthens the approach for evaluating antioxidant effects. Additionally we present the results of a pilot study analyzing NF-κB in human healthy or 'stressed' target tissue (e.g. colon biopts). Moreover we present a method for the analysis of phosphorylated I-κB in the cytosol of mononuclear cells.

References:

[1]Van den Berg et.al. (2001) Br J Nutr 86: S121-S127.

[2]Drent M et.al. (2001) Sarc. Vasc. Diff. Lung Dis. 18: 50-56.

[3]Van den Berg et al. (2001) Environ Tox. Pharm. 9: 147-151.

[4]Van den Berg et. al. (2001) J. Nutr. 131: 1714-1722.

Can We Count Vegetable Soups as a Serving When Considering Daily Vegetable Intake?

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Dietary guidelines recommend a diet rich in fruits and vegetables as part of a healthy life style. However, in spite of intensive promotion activities the daily intake of fruit and vegetables per day is still below the recommendation of five servings in most European countries. Could the consumption of vegetable soups contribute to the daily intake of vegetables? The most straightforward way to do this is to convert the amount of raw vegetables used in the preparation of the soups into the number of servings. This definition does, however, provide little information on the preservation of micronutrients during production process. More detailed information on the micronutrient composition in vegetable soups is desirable as this will further strengthen the idea that processed food can potentially be equally valuable compared to fresh sources. The aim of the present study was to evaluate the antioxidant content and to characterise the antioxidant profile present in vegetable soups. The findings demonstrate that vegetable soups are rich in antioxidants and that all soups investigated provide per serving at least one serving of vegetables based on antioxidant content. Further research is necessary to evaluate the amount of other valuable (micro) nutrients of vegetables in the soups, such as fiber, folate and potassium.

Scientific Substantiation of Health Claims On Foods

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Functional foods are as "foods and beverages with claimed health benefits based on scientific evidence". The regulatory environment for allowing health claims is constantly on the change, and also includes Codex Alimentarius (July 2003; pages 38-42; ftp://ftp.fao.org/codex/alinorm03/al0322Ae.pdf). Recently significant new developments have been published such as by the USA (FDA December 2002; http://cfsan.fda.gov/~dms.hclmgui2.html) and the European Union (EU July 2003; http://europa.eu.int/comm/food/fs/fl/fl07_en.pdf). The existing guidelines all comprise procedural aspects. In all guidelines it is agreed that health claims on foods need to be substantiated scientifically, albeit that in many cases the scientific requirements are not spelled out are not provided at all. As concerns the latter, ILSI Europe currently runs the EU-project PASSCLAIM (Process for the Assessment of Scientific Support for Claims on Foods; http://europe.ilsi.org/passclaim/). PASSCLAIM builds on the basis of the successful EU-project FUFOSE (Functional Food Science in Europe). Within FUFOSE it was identified that claims for "enhanced function" and "reduction of risk of disease" are only justifiable when they are based on appropriate studies using validated markers of exposure, enhanced function or reduction of risk of disease. The aim of the PASSCLAIM project is to develop a set of criteria for the scientific substantiation of claims on foods. The project started in 2001 and will end in 2005. In September 2002 a group of ~80 scientists, regulators, consumers representatives and other stakeholders met for 3 days in Berlin, Germany, to discuss developments and to develop a first set of criteria. The papers have recently been published in the European Journal of Nutrition. This volume comprises the first set of criteria for substantiation of health claims.

Characterization of Reactive Nitrogen/Oxygen species in a co-culture system

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Microglia are the resident immune cells in the central nervous system and upon activation, produce a slew of neurotoxic factors such as RNS/ROS, cytokines and pro inflammatory factors. Accumulating evidence show that RNS/ROS produced by microglia can kill neurons in an *in vitro* system. Addition of lipoplysaccharide (LPS) induces the activation of iNOS and NADPH oxidase, which produce NO and $O_2^{-\bullet}$ respectively into the extracellular milieu. NO and O_2^{-} react at diffusion controlled rates to generate peroxynitrite ($ONOO^-$) which has well established toxicological significance. This has led some to conclude that it is $ONOO^-$ that mediates microglia induced apoptosis in neurons. However, the half life of $ONOO^-$ is approximately 1 second before it decomposes into nitrate while the onset of apoptosis occurs around 24 hrs after exposure to microglia. On the other hand, there are other evidences showing that NO is responsible for inducing apoptosis in neurons. Up to now, the measurement of only nitrite has been carried out to determine if NO is being produced and to what extent. However, the by product of $ONOO^-$ decomposition, nitrate, has not been taken into account. Hence, the goal of this study is an attempt to further characterize the identity of RNS/ROS partaking in neuronal apoptosis and the effects of these RNS/ROS on the microglia itself and neurons in a co-culture system by (1) Determining the nitrite/nitrate ratio, (2) the characterization of RNS/ROS, and (3) viability of neurons and microglia.

Genomic and proteomic profiling of the neuroprotective mechanisms of antiParkinson drug, rasagiline

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Rasagiline is a MAO-B inhibitor antiParkinson¡¦s disease (PD) drug with a possible disease modifying activity, which is now at FDA for approval. Its neuroprotective properties has been examined in many neuronal culture preparations and In Vivo models. These studies have indicated involvement of Bcl-2 family proteins at mitochondrial permeability transition pore (MPT), PKC-dependent MAPkinase pathway and other anti apoptotic genes. To reveal these mechanisms we adopted two novel methods: genomic and proteomic profiling systems, in the mouse model of MPTP. Both RNA, and protein fraction were produced from midbrains of mice, cDNA was synthesized and was hybridized to microarray gene chip (Clontech, CA, USA), containing ~2000 genes, while proteins were detected and analyzed in Powerblot Proteomic array system (BD, CA, USA) containing over 1000 mAbs. Gene analysis demonstrated that MPTP neurotoxicity involves pro-apoptotic mechanism and proteomics indicated that the JNK pathway is recruited by MPTP, in addition to Fas ligand, nNOS, glutamate receptor, and phosphorylated form of GSK-3β. Proteomic analysis confirmed the gene expression profile related to MPTP neurotoxicity Rasagiline reversed the pro apoptotic action of MPTP, enhanced anti-apoptotic Bcl-2, AKT, PKC, growth factor and in particular β-NGF, its downstream transcription factors: EGR1 (NGF-I A binding protein) and EGR3 (early growth response protein-3) gene expressions. Rasagiline's ability to up regulate the protein levels of pro-survival signaling of Ras, PI3K, AKT and PKC indicates that its neuroprotective mechanism activity is partly mediated via neurotrophic factors. Rasagiline has been chosen by NIH as a handful of drugs for neuroprotective studies in Parkinsonian subjects.

4HNE Increases GGT Expression through MAP Kinase Pathways in Rat Alveolar Type 2 Cells

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γ -Glutamyl transpeptidase (GGT) plays key roles in the metabolism of glutathione (GSH) S-conjugates, and in de novo synthesis of GSH. GGT is an essential component of the cellular adaptive response, and its expression is increased during oxidative stress, but the mechanism of this up-regulation remains unclear. In rats, GGT is a single copy gene regulated by 5 tandemly-arranged promoters; alternative splicing allows for 7 different transcripts. Our previous studies have shown that 4-hydroxy-2-nonenal, an end product of lipid peroxidation, upregulated GGT transcription in rat lung alveolar type II (L2) cells, and that mRNA subtypes I and V were involved. Previously we demonstrated that 4HNE activated the ERK, p38 and JNK MAPK signaling pathways in L2 cells. In the present study, we investigated which MAPK signaling pathways are involved in GGT up regulation by 4HNE in L2 cells. PD98059 and SB203580, specific inhibitors of the ERK and p38 pathways, respectively, blocked the 4HNE-mediated up-regulation of both GGT activity and mRNA; surprisingly, SP600125, which specifically inhibits JNK1/2, synergistically increased the induction of GGT by 4HNE. In conclusion, our results demonstrate that the signaling for increasing GGT expression in response to 4HNE is mediated through MAP kinase pathways in rat alveolar type II cells.

Effect of JNKs in Mitochondria Meditated Neuronal Apoptosis

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JNKs, a subfamily of MAPKs, consist of at least ten isoforms derived from the alternative splicing of three related genes, Jnk1, Jnk2, and Jnk3. Jnk1 and Jnk2 are widely expressed in adult tissues, Jnk3 is mainly expressed in the nervous system. Existing evidence indicates that different Jnk isoforms have distinct biological functions. Previous studies strongly suggest an active involvement of the JNK signaling pathway in mitochondria mediated neuronal apoptosis. The direct evidence for the role of JNK and its isoforms has been missing and the mechanism of how JNK actually could facilitate this process has remained unclear. Our previous study showed that in vitro, JNK modulates mitochondria function via phosphorylation of Bcl-2 and Bcl-XL and subsequent changes membrane potential. In this study, we showed: (a). The modulation JNK isoform levels in rat brain by aging. (b). The interaction of JNK with mitochondrion to modulate its function. (c). JNK-mediated phosphorylation of mitochondrial proteins other than Bcl-2 and Bcl-XL by *in vitro* assay.

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