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OXIDANTS AND ANTIOXIDANTS IN BIOLOGY

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OXIDANTS AND ANTIOXIDANTS IN BIOLOGY

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CONTENTS

KEYNOTE ADDRESSES

Mechanisms of delivery of metabolites and drugs across the blood-brain barrier

William M. Pardridge

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Nutrients and pharmaceuticals are excluded from entering brain by the brain capillary endothelial wall, which forms the blood-brain barrier (BBB) in vivo. Circulating molecules gain access to the brain only via 1 of 2 mechanisms: lipid-mediated free diffusion or catalyzed (mediated) transport. The number of molecules that cross the BBB via lipid-mediated free diffusion is surprisingly small, because only molecules that are both lipid-soluble and have a molecular weight (MW) less than 400 Daltons (Da) are able to penetrate the BBB via free diffusion. Molecules that are either water soluble or have MW >400 Da may cross the BBB via catalyzed transport. There are 3 broad classes of BBB catalyzed transport: carrier-mediated transport (CMT) for small molecules, active efflux transport (AET) for small molecules, and receptor-mediated transport (RMT) for large molecules. The CMT systems include the GLUT1 glucose transporter, the LAT1 large neutral amino acid transporter, and many other membrane transporters with affinity for molecules with defined molecular structure. The AET systems include p-glycoprotein, organic anion transporting polypeptide type 2 (oatp2), and many other systems, which serve to actively pump small molecules from brain back to blood. Some molecules influx from blood to brain via lipid-mediated free diffusion, but are actively effluxed from brain to blood via one of the BBB AET transporters. The RMT systems include the insulin receptor, the transferrin receptor, the leptin receptor, and others. Circulating insulin gains access to the brain via RMT on the BBB insulin receptor. Similarly, peptidomimetic monoclonal antibodies against the insulin receptor also are transported, and act as molecular Trojan horses for brain drug delivery.

Sensory integration across space and in time: Grey matter(s)

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Decision making relies on sensory input, comparison with previous experience, and motor action. A simple rodent behaviour is reward-driven gap crossing. It can rely on the sensory input from a single vibrissa and exciting a single column in the barrel cortex. The major output emitted by a single column is by the thick-tufted pyramids in the infragranular layer that project to the pons. Possibly, these pyramids establish the link between sensory input and motor action.

SESSION I
AGE-REGULATED METABOLIC PATHWAYS, MITOCHONDRIAL NUTRIENTS, AND NEURODEGENERATION

Lipoic acid and acetylcarnitine supplementation delay mitochondrial decay and cognitive dysfunction

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Mitochondrial decay appears to be a major contributor to aging and associated degenerative diseases. Aging mitochondria exhibit a decrease in membrane potential, respiratory control ratio, cardiolipin, and cellular oxygen consumption, and an increase in oxidant by-products. Oxidative damage to DNA, RNA, proteins, and lipids in mitochondrial membranes is a major consequence of this decay, resulting in functional decline of mitochondria, cells, and organs such as the brain. Feeding the mitochondrial metabolites acetyl carnitine and lipoic acid (LA) to old rats rejuvenates the mitochondria and improves brain and other function. Recent studies on dogs show that the combination also improves cognition in old beagle dogs. A recent double blind clinical trial on aged humans from Dr. Vita's laboratory shows a decrease in hypertension. Supported by extensive preliminary results showing that the combination lowers C-reactive protein in sickle cell disease, a clinical trial on the combination was recently funded to test the effect of the combination on inflammation accompanying sickle cell disease. Lipoic acid has also been shown to protect retinal pigment epithelial cells from oxidation, a model for macular degeneration. LA increases the lifespan of human cells in culture and is known from work in the Hagen's lab to induce the ~250 phase II antioxidant protective enzymes including those for GSH synthesis.

Loss of mitochondrial NAD(H) and pyruvate dehydrogenase impair respiration after cerebral ischemia and reperfusion

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Compared to normoxic resuscitation after global cerebral ischemia, hyperoxic reperfusion worsens neurologic outcome by promoting oxidative stress and impairing cerebral energy metabolism. Using an animal model of cardiac arrest and resuscitation, the level and labeling pattern of metabolites derived from ^{13}C-glucose present in the hippocampus of hyperoxic animals at 2 hr reperfusion indicate impaired flux through both pyruvate dehydrogenase complex (PDHC). Although we also observed hippocampus-selective inhibition of PDHC activity and immunoreactivity, mitochondrial respiratory impairment was evident with other NAD-linked substrates, suggesting additional mechanisms. Measurements of total cellular pyridine nucleotide (PN) levels and fluorescent imaging of PN redox state indicate that oxidative stress and glucose deprivation promote PARP-1 mediated neuronal NAD^+ catabolism. Mitochondria isolated from the brains of neonatal rats after 75 min of hypoxia/ischemia and 30 min reoxygenation also exhibit reduced NAD^+ levels and respiratory impairment that is fully reversible by addition of exogenous NAD^+ (but not NADH). In summary, postischemic oxidative stress impairs brain mitochondrial energy metabolism by both inactivation of PDHC and by inducing the release and catabolism of NAD(H). These pathologic events can be overcome by several neuroprotective interventions, including avoiding hyperoxia and addition of exogenous NAD^+.

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Lipoic acid in Alzheimer's disease – From basic therapeutic concepts to clinical trials

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Alzheimers disease (AD) is a progressive neurodegenerative disorder that destroys patient memory and cognition and the ability to carry out daily activities. Despite extensive research into the pathogenesis of AD, a neuroprotective treatment remains unavailable. LA has a variety of properties, which might be able to interfere with pathogenic principles of AD. For example, LA increases glucose uptake, chelates redox-active transition metals and scavenges reactive oxygen species (ROS. Since AD is characterized by chronic inflammation, reduction of pro-inflammatory cytokine expression and free radical production by interference with redox-active signalling by LA might be beneficial for AD patients. In a cell culture model of brain inflammation, we show that LA dose-dependently reduces LPS+IFN-gamma induced nitric oxide production in N-11 murine microglia, with the two enantiomers and the RS form being equally active. In an open clinical trial, 43 patients were given 600mg RS-LA for 48 months. Whereas LA did not show significant benefits in moderate and severe AD, cognitive scores in LA treated patients with early dementia (ADAScog<15) deteriorated significantly less than in the control group only treated with cholinesterase-inhibitors (ADAScog: 1.2 points/year vs. 2.6 points/year in untreated controls, MMSE: -0.6 points/year vs. -2 points/year in untreated controls). Despite the fact that this study was an open trial, our data suggest that LA acid might become a standard 'neuroprotective' therapy option for AD.

Oxidative stress in Alzheimer's disease brain and models thereof: *In vivo* neuroprotection by multifunctional, brain-accessible antioxidants

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Our group reported elevated oxidative stress in brain from subjects with Alzheimer's disease (AD) and with mild cognitive impairment (MCI), arguably the earliest form of AD. We also showed that amyloid β-peptide 1-42 (Aβ42) could induce oxidative stress in brain *in vivo*. We described the first use of proteomics to identify oxidatively modified brain proteins in AD and MCI. Several proteins were identified to be oxidatively modified in common in AD and MCI, suggesting a key role for these proteins and their associated biochemical pathways in the progression of AD. Aβ42 in various model systems *in vivo* is able to replicate many of these proteomics-identified oxidatively modified brain proteins in AD and MCI, pointing to this peptide as a major contributor to the oxidative stress inherent in both disorders. Three examples: (a) old beagles deposit Aβ42 of the same sequence as humans in brain and demonstrate dementia. Old dogs treated with antioxidants and a program of behavioral enrichment (synapse formation) led in brain to loss of oxidative stress and lower levels of Aβ42, and led to enhanced performance on memory tests similar to young dogs. (b) Rodents were injected i.p. with the ethyl ester of ferulic acid; subsequent analysis of Aβ42-induced oxidative damage to synaptosomes showed marked protection. FAEE induces HSP70 and HSP32 while down regulating i-NOS. (c) Injection i.p. of the glutathione mimetic, D609, into rodents protects subsequently isolated brain mitochondria against Aβ42-induced oxidative damage and apoptosis. These results support the use of endogenous and exogenous, brain-accessible, multi-functional antioxidant agents to slow the progression of AD.

Support: NIH-NIA grants.

Translating estrogen mechanisms of action into neuroSERMs and phytoSERMs for prevention of neurodegenerative disease

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Our scientific endeavors are a hybrid of basic science discovery and preclinical translational research. The goal of our basic science discovery is to elucidate fundamental cellular mechanisms of *1*) neural defense and repair and *2*) neural plasticity required for cognitive function. Our therapeutic development goal is to translate our cellular mechanistic insights into safe and efficacious therapeutics for the prevention of and rehabilitation from neurodegenerative diseases, such as Alzheimer's, Parkinson's and stroke. To achieve these goals, we have investigated the mechanisms and neurobiological outcomes of estrogens, progestins, hormone therapies and neurosteroids. Results of these analyses have yielded insights into cellular strategies required for neural defense against degenerative insults that involve multifaceted cytoplasmic and nuclear signaling cascades that converge upon the mitochondria. Further, these signaling cascades are required for gonadal hormone regulation of morphogenesis and neurogenesis. Our data indicate a healthy cell bias of estrogen action for estrogen-inducible neuroprotective and neurotrophic outcomes. Our translational therapeutic development efforts target sites of estrogen action that promote neural defense and plasticity in brain while reducing untoward effects in reproductive tissue. Our novel NeuroSERM molecules are designed to target the membrane site of estrogen action whereas our natural source PhytoSERMs are designed to target estrogen receptor beta. Results of our *in vitro* analyses indicate that our novel NeuroSERM candidate molecule induces neuroprotective efficacy comparable to 17β-estradiol. In addition, NeuroSERM candidate molecule induces neuronal plasticity markers consistent with 17 β-estradiol. In parallel, PhytoSERM formulations show a high degree of estrogenic efficacy in cultured hippocampal and cortical neurons and induces both neuroprotective and neural plasticity reponses. Results of *in vitro* analyses provide proof of principle that combination of ERb selective NeuroSERM and PhytoSERMs provide neuroprotective efficacy, which will be extended into in vivo analyses. The data thus far suggest that novel NeuroSERM molecules and select combinations of PhytoSERMs have the potential to be effective and safe estrogen alternative therapies for preventing estrogen deficiency-related cognitive decline and vulnerability to neurodegenerative insults such as those leading to Alzheimer's disease in postmenopausal women.

This work was supported by NIH (1RO1 MH67159-01 to RDB 7 JN), the Alzheimer's Association (to LZ), the Kenneth T. and Eileen L. Norris Foundation (to RDB) and the Bensussen Translational Research Gift (to RDB)

Targeting mitochondrial reactive oxygen species generation in astrocytes: its enhancement and prevention

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Astrocytes, the most abundant cells in the central nervous system, play crucial role in neuron survival via their active roles in regulating synaptic transmission and neurovascular coupling. With the application of fluorescent probes coupled with laser scanning multi-photon imaging microscopy, our previous studies visualized enhancement of mitochondria-derived reactive oxygen species (mROS) formation upon oxidative stresses induced by oxidants exposure and mtDNA mutation-induced respiratory chain defects. Intriguingly, enhancement of mROS generation also can be specifically targeted by visible light irradiation. Visible light or laser irradiation induced temporal-spatially heterogeneous generation of mROS among different mitochondrial populations within the same cells which propagates rapidly from irradiated mitochondria to non-irradiated adjacent mitochondria and even cells. Light-induced mROS is associated with a secondary mitochondria Ca^{2+} (mCa^{2+}) stress, which is dependent on extracellular Ca^{2+} and mitochondrial uniporter Ca^{2+} uptake. Elevated mROS then cause apoptotic mitochondrial swelling, mitochondrial membrane potential depolarization, mitochondrial permeability transition, cytochrome c release, caspase 3 activation and condensation and fragmentation of nuclei. Mitochondria-targeted protection brought by mito-Q (a mitochondria-targeted antioxidant), melatonin (via multiple mitochondria-targeted mechanisms) and over expression of Bcl-2 (a multiple antiapoptotic protein) prevented significantly the visible laser-induced mROS-targeted apoptosis. Vast augmentation of mROS can be achieved by coupling visible laser irradiation with mitochondria-targeted photosensitizers such as Benzoporphyrin derivative (BPD-MA), nanoparticles (C60 and quantum dots) and a mitochondrial benzodiazepine receptor-targeted photosensitizer (HPPH-In). These novel strategies of mROS targeting may provide great therapeutic impact in the future treatment of glioma and astrocyte-associated neurodegeneration.

Oxidation and assembly of amyloid β-protein in Alzheimer's disease: casual or causal?

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Historically, the pathogenesis of amyloidoses has been linked to amyloid fibril formation. In Alzheimers disease (AD), this linkage has been the central feature of the amyloid cascade hypothesis, which suggests that AD results from a cascade of pathologic events beginning with fibril formation by the amyloid β-protein (Aβ). In the last decade, however, a new hypothesis has emerged that might be called the oligomer cascade hypothesis and that posits that the proximate pathogenetic agent in AD is the Aβ oligomer. In addition, increasing evidence suggests that the ability of Aβ to mediate redox chemistry may be a factor in brain injury in AD. Interestingly, a center for redox chemistry within the 42-residue long Aβ peptide is the amino acid Met35. Oxidation of the Met35 side-chain to its sulfoxide or sulfone form profoundly alters Aβ assembly. Aβ thus may be both a mediator and a target of redox chemistry, a chemistry that can alter Aβ assembly as well as damaging cellular proteins, lipids, and nucleic acids. Whether Aβ oxidation causes AD or results from AD remains enigmatic.

SESSION II
CHOLINE, METALS, AMINO ACIDS, AND LIPOPHILIC MICRONUTRIENTS IN BRAIN HEALTH AND FUNCTION

Choline and brain development

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Choline is an essential nutrient in humans, needed for the structural integrity of cell membranes; for normal cholinergic neurotransmission; and as the major source of methyl-groups in the diet. This nutrient is derived not only from the diet, but as well from de novo synthesis. The gene for the enzyme (PEMT) that catalyzes endogenous choline biosynthesis is induced by estrogen. Almost half the population has a single nucleotide polymorphism in PEMT that makes them insensitive to induction by estrogen, and thereby more dependent on dietary intake. The amount of choline eaten in the diet varies at least two-fold in the US, and this difference in intake is enough to increase by 4x the risk that a women will have a baby with a neural tube defect. Women evolved to have increased capacity for endogenous synthesis of choline because this nutrient is critical during fetal development, when, via epigenetic mechanisms (DNA methylation and histone methylation), it influences neural progenitor cell proliferation and apoptosis in the fetal hippocampus. This results in permanent changes in hippocampal structure and function. The effect of choline on hippocampus is limited to a critical period (in the mouse days 12-17 of gestation) when the primordial hippocampus is formed. Extra choline during this period results in a 30% enhancement in spatial memory that lasts lifelong. Too little choline during this period results in decreased memory function. Restoration of choline after the critical period does not reverse the deficit.

This work was funded by a grant from the National Institutes of Health (DK55865, AG09525) and the Gerber Foundation. Support for this work was also provided by grants from the NIH to the UNC Clinical Nutrition Research Unit (DK56350), the UNC General Clinical Research Center (RR00046) and the Center for Environmental Health and Susceptibility (ES10126).

Excitatory amino acids, free radicals, and protein misfolding in neurodegenerataive disease: protection by memantine and nitro-memantine at NMDA-gated channels

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Excitotoxicity has been implicated in a final common pathway contributing to neuronal injury and death in a wide range of acute and chronic neurologic disorders. Excitotoxic cell death is due, at least in part, to excessive activation of NMDA-type glutamate receptors, leading to excessive Ca^{2+} influx through the receptor's associated ion channel and subsequent free radical production, including nitric oxide (NO) and reactive oxygen species (ROS). Fulminant insults can result in necrotic-like cell death, while more subtle insults can produce synaptic injury and apoptotic-like damage. These free radicals can trigger a variety of injurious pathways, but newly discovered evidence suggests that some proteins are S-nitrosylated (transfer of NO to a critical thiol group to regulate protein function), and this reaction can mimic the effect of rare genetic mutations that cause disease. For example, this posttranslational modification can herald protein misfolding, and thus contribute to neurodegeneration. One such molecule affected is protein-disulfide isomerase (PDI), an enzyme responsible for normal protein folding in the ER. We found S-nitrosylation of PDI (forming SNO-PDI) compromises enzyme function, leading to misfolded proteins, neuronal injury, and death. Moreover, SNO-PDI occurs at pathologically relevant levels in human conditions, including Alzheimer's and Parkinson's diseases. This discovery links protein misfolding to excitotoxicity and free radical formation in a number of neurodegenerative disorders. Blockade of NMDAR activity can, in large measure, protect neurons from this type of injury if Uncompetitive/Fast Off-rate (UFO)-type therapeutics are employed because these drugs block excessive, predominantly extrasynaptic NMDAR activity without disrupting normal synaptic activity.

Zinc and the cytoskeleton in the neuronal modulation of transcription factor NFAT

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Zinc is a key micronutrient in the physiology of the nervous system. We investigated if the alterations in neuronal cytoskeleton associated with a low Zn availability could affect select transcription factors (NFAT and NF-κB) that are activated in the cytosol and then translocated into the nucleus. In human neuroblastoma IMR-32 cells, a decrease in cellular Zn was associated with the oxidant-dependent triggering of the initial (cytosolic) events of the NFAT cascade. However, the active NFAT accumulated in the cytosol, the nuclear transport was impared, and a decreased NFAT-dependent gene expression was observed. The requirement of a functional cytoskeleton for NFAT modulation was supported by the altered nuclear transport associated with the cytoskeleton disruption induced by compounds that affect tubulin and actin polymerization. Similar findings were observed for NF-κB in neuronal cells, and for both transcription factors in the brain of zinc deficient rats. Prevention of zinc deficiency-induced oxidant increase by lipoic acid normalizes tubulin polymerization and restores NF-κB nuclear transport. In summary, a decreased Zn availability leads to an increase in neuronal oxidants, alterations in cytoskeleton dynamics, and in NFAT and NF-κB signaling. Given the role of these transcription factors in cell proliferation, differentiation, and apoptosis, a low Zn availability during critical periods could affect neurodevelopment.

This work was supported by grants from University of California, Davis, and NIH (HD 01743).

AVED mouse:
An *in vivo* model for neuroprotective actions of α-tocopherol

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Ataxia with vitamin E deficiency (AVED) is a neurological disease caused by mutations in alpha-tocopherol transfer protein (ATTP) gene and can be ameliorated by alpha-tocopherol (AT) supplementations. The brain and other tissues of AVED patients are severely deficient in AT. The biochemical and ataxic phenotype is reproduced in mice by the inactivation of ATTP gene (ATTP-KO). The age-dependant ataxic phenotype of ATTP-KO mice can be prevented by dietary supplementation of AT (350 IU AT/Kg diet). Thus, the AVED-mouse offers an in vivo model to uncover mechanisms of AT-actions, and possibly of other tocopherols, tocotrienols and micronutrients in the prevention of age-related decline in neuromuscular functions. We have analyzed data for hematological parameters, liver function tests under normal and viral challenge, unstressed cardiac functions, organ weights, histological parameters of eyes, brains and muscles, and neurological functions in at least two cohorts of ATTP-KO mice and their congenic controls. The most striking and statistically significant differences were noted for tissue AT levels, neurological tests and muscle histology. AT levels in five different brain regions of ATTP-KO mice were less than 5% of that in the controls. Ataxia developed at about nine months of age in spite of severe and early, possibly at birth, brain AT-deficiency. Muscle histology was abnormal. Genome-wide analysis of mRNAs and microRNAs, and analysis of proteins by 2D-MALDI identified remarkable differences between the ATTP-KO and control mice in the expression of these macromolecules. Transcriptomic analysis identified distinct responses to AT-deficiency in cortex and cerebellum. Five overexpressed proteins identified by 2D-MALDI suggest deregulated calcium sensing in cerebella of ATTP-KO mice. These experiments identify novel molecular actions of AT that are brain region specific and that appear to converge or act on polymerase II and the translational machinery.

Stroke: Tocotrienol function and a novel pre-clinical model for translational research

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The tocotrienol (T3) form of vitamin E is poorly studied and has, for decades, been overshadowed by the tocopherols. With clinical trials of α-tocopherol providing disappointing results current interest is being refocused on the non-α-tocopherol forms of vitamin E. Long before this current wave of change, seven years ago our laboratory noted that nanomolar α-T3, but not α-tocopherol, blocked glutamate-induced neuronal death by suppressing early activation of c-Src kinase (*J Biol Chem* 275:13049, 2000; 282(32):23482-90, 2007). A later independent study reporting that Src blockade provides cerebral protection following stroke (*Nature Med* 7:222, 2001) enhanced the significance of our finding that α -T3 possesses c-Src regulatory effects. Our efforts to understand the mechanistic basis of neuroprotection by α-T3 have led to the observation that glutamate-induced neurodegeneration hinges on two key molecular checkpoints: (i) c-Src activation, and (ii) 12-lipoxygenase (Lox) activation (*JBC* 278:43508, 2003). We also noted that *in vivo* α-T3 supplementation decreased stroke-induced damage to the brain of spontaneously hypertensive rats (*Stroke*, 36:2258, 2005). A preclinical model of canine stroke has been recently developed by our laboratory to test the efficacy of α-T3 in a large animal system that closely approximates that of humans. Using a minimally invasive interventional radiological approach, a platinum coil is directed to occlude the middle cerebral artery (MCA) for 1h under guided fluoroscopy. Following real-time visualization of occlusion, the coil is retrieved to model reperfusion after acute focal ischemia. In a small pilot study (n=5), the mean percent hemispherical infarct volume as determined by 3T MRI at 24h post-reperfusion was 28.7±5.3. This highly reproducible endovascular approach of transient MCA occlusion serves as a robust preclinical stroke model to study the neuroprotective properties a-T3 and to overcome what the NIH has identified as "the translational barriers that exist today in stroke research". α-T3 seems to protect neurons both by antioxidant-independent and –dependent mechanisms (*J. Neurochem* 98:1474, 2006). Taken together, orally supplemented α-T3 is potently neuroprotective and should be considered for preclinical and clinical testing (*Vitam Horm* 76:203-61, 2007).

The phenotype of the taurine transporter knockout mouse

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Taurine is abundant in many cell types, where it is accumulated by the taurine transport system (TauT). Taurine is involved in cell volume homeostasis, antioxidant defense, protein stabilization, stress responses and immunomodulation. In order to test the hypothesis that taurine deficiency may predispose to organ damage, we have generated a TauT (taut-/-) knockout mouse by deletion of exon 1 (FASEB J. 2002; 16,231-233). These animals exhibit decreased taurine levels in skeletal and heart muscle and skin by about 98%, by 80-90% in brain, kidney, plasma and the retina, whereas hepatic taurine levels are decreased by about 70% only. Taut-/- mice exhibit reduced fertility and develop within the first 6 weeks post partum severe retinal degeneration due to photoreceptor cell apoptosis. Also the auditory and olfactory system becomes functionally compromised. TauT knockout produces severe skeletal muscle dysfunction, whereas cardiac function was largely uncompromised, possibly due to an osmotic compensation of taurine deficiency by amino acids in heart, but not in skeletal muscle (FASEB J. 2004; 18,577-579). However, compensated cardiac stress is suggested by the fact that atrial and brain natriuretic peptides were upregulated. Taurine levels were strongly reduced in Kupffer cells and sinusoidal endothelial cells, but not in liver parenchymal cells. Nonetheless, TauT knockout mice exhibited increased hepatocyte apoptosis and signs of mild hepatitis and fibrosis (FASEB J. 2006; 20, 574-576). This was accompanied by increased levels of tumor necrosis factor-α, oval cell proliferation and mitochondrial dysfunction. The skin of TauT knockout mice showed a significantly higher sensitivity to UV-B-induced immmunosuppression due to an enhanced formation of platelet activating factor and interleukin-10. Taurine deficiency triggers organ damage due to increased oxidative stress, mitochondrial dysfunction and overshooting immune responses.

SESSION III
FLAVONOIDS IN CELL SIGNALING AND NEURONAL FUNCTION

Modulation of multiple pathways involved in the maintenance of neuronal function by fisetin

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The pathology of neurological disorders is complex and appears to affect multiple cellular functions. Because of this complexity, it has been suggested that multiple drugs may be needed to reduce or prevent the nerve cell dysfunction and death that are characteristics of these disorders. An alternative approach is to identify compounds with multiple biological activities relevant to the treatment of neurological disorders. Over the last few years, we have identified an orally active, novel neuroprotective and cognition-enhancing molecule, the flavonoid fisetin. Fisetin not only has direct antioxidant activity but it can also increase the intracellular levels of glutathione, the major intracellular antioxidant. Fisetin can also activate the Ras-ERK cascade and activation of this signaling pathway is associated with the neuroprotective, neurotrophic and cognition enhancing effects of fisetin. In addition, fisetin can activate several transcription factors associated with both the protection of neural cells from stress and the maintenance of neuronal function. Among the transcription factors activated in neural cells by fisetin are NF-E2-related factor 2 (Nrf2), ATF4 and cAMP response element binding protein (CREB). Thus, by inducing the expression of a variety of genes and their protein products, fisetin has the potential to have long-lasting effects on neural function. Very recently we have found that fisetin can increase proteasome activity and therefore might be able to reduce the accumulation of toxic proteins that are associated with a variety of neurological disorders. Fisetin also possesses anti-inflammatory activity and we have recently found that it can inhibit lipoxygenases, thereby reducing the production of lipid peroxides and their pro-inflammatory byproducts. Thus, this multiplicity of activities suggests that fisetin has the potential to reduce the broad array of metabolic dysfunctions that are associated with neurological disorders.

Do flavan-3-ols from green tea reach the human brain?

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Two hundred and fifty mL of green tea was fed to three male and six female subjects with suspected multiple sclerosis. Venous blood samples were collected after 1 h and cerebrospinal fluid after three hours. The principal flavan-3-ols in the green tea were (–)-epicatechin (53 µmole), (–)-epigallocatechin (149 µmole), (–)-epigallocatechin gallate (206 µmole) and (–)-epicatechin gallate (97 µmole). Analysis of plasma by HPLC with tandem mass spectrometric (MS^2) detection revealed the presence of a number of (epi)catechin glucuronide, methyl and sulfated metabolites at an overall concentration of 1.56 ± 0.77 µmole/L. However, despite extensive HPLC-MS^2 analysis in the full scan and selected ion monitoring modes, flavan-3-ols metabolites were not detected in extracts of the cerebrospinal fluid collected from any of the six subjects. These data will be discussed along an evaluation of studies with animal models, by other investigators, in which evidence is presented indicating that anthocyanins and flavan-3-ols are able to cross the blood-brain-barrier.

Molecular mechanisms underlying the cognitive effects of polyphenols

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Flavonoids may exert beneficial effects in the brain by protecting neurons against stress-induced injury, by suppressing neuroinflammation, and by promoting neuro-cognitive performance. We show that supplementation with a blueberry diet improves the performance of aged animals in spatial working memory tasks within 3 weeks. Spatial memory task performance correlated with a specific activation of cAMP-response element-binding protein (CREB) and an increase in both pro- and mature levels of brain-derived neurotrophic factor (BDNF) in the hippocampus. Changes in CREB and BDNF appeared to be mediated by increases in the phosphorylation state of extracellular signal-related kinase (ERK1/2), rather than that of calcium calmodulin kinase or protein kinase A. We also observed increases in the activation state of Akt, mTOR and the levels of Arc/Arg3.1 in the hippocampus, suggesting that blueberry supplementation induces pathways involved in *de novo* protein synthesis linked to changes in neuronal morphology. In a follow up acute human intervention trial, supplementation of a blueberry-rich drink induced a significant improvement in attentional performance, a measure of executive function. Specifically, participants displayed a more sustained ability to correctly detect target stimuli following flavonoid supplementation compared to the placebo treatment ($p = 0.03$; $n = 12$).

Estrogen or genistein prevent β-amyloid-induced cell death by inhibiting the p38 pathway

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Estrogenic compounds have been postulated as neuroprotective agents. This prompted us to investigate their mechanism action in neurons in primary culture. Cells were pre-treated with estradiol or with genistein and 48 hours later treated with beta amyloid. We found that beta amyloid increased oxidative stress which in turn, caused phosphorylation of p38 MAP kinase and subsequently induced neuronal death. All these effects were prevented by oestradiol or genistein. Since hormone replacement therapy with estradiol has serious setbacks, the potential therapeutic effect of phytoestrogens for the prevention of beta amyloid associated neurodegenerative disorders should be more carefully studied in clinical research.

Tea polyphenols and prostate cancer

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Green and black teas contain gallated ((-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin-3-gallate (ECG)) and non-gallated ((-)-epicatechin (EC), (-)-epigallocatechin (EGC)) tea polyphenols. During black tea production polyphenols (PP) undergo oxidation and form larger polymers such as theaflavins (THE) and thearubigins (THR). Epidemiological and phase II intervention studies provide evidence that the consumption of green tea delays the development of prostate cancer. In addition to their antioxidant activity it has been demonstrated that green tea PPs decreased the protein concentration of phospho-Met, phospho-AKT in prostate tissue as well as serum PSA, HGF and VEGF in a human phase two intervention study. The chemical characteristics and endogenous metabolism of tea polyphenols are important factors in their bioavailability and anticarcinogenic activity. At neutral pH EGCG and EGC form homo and heterodimers generating hydrogen peroxide. This may decrease their concentration in the small intestine and interfere with cell culture experiments. The metabolism of tea PPs includes conjugation, enterohepatic circulation, and colon microflora transformation resulting in phenolic acids. Human tea intervention studies determined that gallated tea PP are more bioavailable compared to non-gallated PP and are present in the highest concentration in plasma, urine, and prostate after the administration of green tea, black tea or a green tea supplement. An in vitro colon simulation study determined the formation of EGC from black tea powder and determined that the same phenolic acids were formed during the digestion of green and black tea. Future intervention studies will need to focus not only on the bioactivity of green and black tea polyphenols but also their metabolites and biotransformation products.

Metabolites and molecular targets of pomegranate ellagitannins

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Pomegranate juice (PJ) ellagitannins with marked antioxidant and anti-inflammaotry effects are hydrolyzed in the gut leading to a prolonged rise in plasma ellagic acid (EA) over 5 hours in humans. Urinary EA metabolites arise from intestinal metabolism of EA and subsequent conjugation and methylation in liver and excretion in the urine. After a single serving of PJ, dimethylellagic acid glucuronide is found in urine for 24 hr. while urolithins are found in urine for up to 56 h. Urolithin A is widely distributed in tissues concentrating in the prostate and colon while methylated UA is found in brain tissue. Inflammatory cells and ROS are known to activate NF-κB which induces the expression of multiple mediators of inflammation including COX-2, 5-LOX, and cytokines such as IL-6 and in so doing may further promote the inflammatory process leading to a cycle of inflammation and NF-κB activation followed by further inflammation. PI3K is downstream of growth factor receptors, such as epidermal growth factor receptor (EGFR). Signals activated by PI3K are mediated largely through protein kinase B (AKT) which in turn can activate downstream mediators such as mTOR. AKT is also a key regulator of NF-κB activity. One mechanism whereby Akt can regulate NF-κB activity is through its ability to modulate phosphorylation of IκB kinase a (IKKa) protein complex. We have preliminary data which demonstrates that PJ can inhibit TNF-α -induced NFκB activation by inhibiting phosphorylation of IKBα in prostate cancer cells. We are currently studying the interaction of these two major signaling pathways in prostate cancer and colon cancer but suggest that these pathways may mediate effects of PJ for many different common chronic diseases of aging.

Pomegranate actions in Alzheimer's disease pathophysiology

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The development of Alzheimer's disease (AD) is likely related to the age- related buildup of a protein (Aβ) in the brain. Cognitive deficits do not appear until significant brain pathology has accumulated. Evidence suggests that diet can alter concentrations of Aβ in the brain and the risk of developing AD. A high-cholesterol diet is associated with increased risk, and cholesterol elevates brain Aβ levels in animal models of AD. Evidence also suggests that diet can decrease the risk of AD. Regular intake of fruits and vegetables is associated with decreased risk, and diets high in bioactive phytochemicals (e.g., polyphenols) reduce brain Aβ levels in animal models of AD. Pomegranates contain relatively high levels of polyphenols, and pomegranate juice (PJ) reduced brain damage in a mouse model of stroke. To test whether PJ could ameliorate age-related Aβ accumulation and/or cognitive deficits, transgenic (AD) mice were given dilute PJ in their water bottles from 6-12 months of age. These mice generally develop Alzheimer's-like Aβ deposits in their brains along with learning/memory deficits at 7-9 months of age. Daily consumption was roughly equivalent on a L/g basis to an adult human drinking 250-500 ml of full strength PJ. Mice that drank PJ had ~50% less soluble Aβ and amyloid plaques in their brains compared to controls. They also learned cognitive tasks quicker and swam faster. Thus, daily intake of PJ prevented or delayed the development of both Alzheimer's-like neuropathology and behavioral deficits. Because AD is a disease of aging, delaying the onset by only a few years would significantly decrease its prevalence. These data provide preclinical evidence that a diet incorporating pomegranates and/or other foods with high polyphenolic content may help to maintain and protect brain health throughout life.

SESSION IV
OXIDATIVE STRESS AND THIOL REDOX CIRCUITS IN CELL FUNCTION

Oxidative stress and thiol redox circuits

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Cysteine residues of proteins serve as reversible sulfur switches which function in redox signaling and control of macromolecular structure and function. Dynamic regulation of these control elements occurs through thiol redox circuits which are driven by oxidative mechanisms dependent upon O_2, H_2O_2 and other oxidants, and counterbalanced by NADPH-dependent reduction pathways. Disruption of these thiol redox circuits is a common feature of oxidative stress. GSH/GSSG and thioredoxin systems serve as central control nodes for redox pathways in cells, while Cys/CySS provides a central redox couple in extracellular fluids. The redox control nodes are maintained at stable but non-equilibrium states, differing with respect to subcellular compartment and pathophysiologic state. In general, the couples vary, from most reduced to most oxidized, in the following sequences: Trx, GSH, Cys and mitochondria, nuclei, cytoplasm, secretory pathway, extracellular fluids. The range of steady-state redox potentials is from -360 mV for thioredoxin-2 (Trx2) in mitochondria to -20 mV for Cys/CySS under pathologic conditions in human plasma. Because a 30-mV change is sufficient for a 10-fold change in dithiol/disulfide ratio, this range is ample for considerable specificity in redox control of different protein thiols. Measurement of steady-state reduction in the mitochondrial pathway: NADPH, Trx reductase-2, Trx2, peroxiredoxin-3; and the nuclear/cytoplasmic pathway: NADPH, Trx reductase-1, Trx1, Ref1, NF-κB p50 subunit, show that these pathways are kinetically limited as they function in cells. These observations provide an initial framework for development of redox systems biology descriptions of thiol redox circuits and their disruption in oxidative stress. Importantly, this framework identifies critical needs to understand the insulation and communication of different redox circuits, to delineate the electron transfer pathways controlling specific sulfur switches and to discriminate orthogonal redox regulatory mechanisms, which appear to provide the basis for “cross-talk” between pathways.

Keap1/Nrf2 system as thiol-based sensor/effector machinery for cytoprotection

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Nrf2 is a key regulator of many detoxifying enzyme genes, and cytoplasmic protein Keap1 represses the Nrf2 activity under quiescent conditions. In response to electrophilic or oxidative stress, Nrf2 is derepressed and activates target genes by heterodimerizing with small Maf proteins. To clarify the molecular mechanisms of Nrf2 activation, we performed structural analyses of interacting domains of Keap1 and Nrf2 and proposed ghinge and latchh model. We then examined the in vivo significance of Keap1 BTB domain and several cysteine residues for regulating the Nrf2 activity by exploiting transgenic mouse complementation rescue approach. The results indicated that BTB domain is indispensable to repressor activity of Keap1, and demonstrated the functional importance of the cysteine residues; two cysteine residues C273 and C288 in the intervening region are involved in the maintenance of Keap1 repressor activity and one cysteine C151 in BTB domain is involved in Keap1 reactivity to electrophilic reagents. Current questions and future assignments will be also discussed.

Effects of oxidation and nitric oxide donors on mammalian thioredoxins and glutaredoxins and their targets

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Mammalian thioredoxin and glutaredoxin exist in isoforms located in the cytosol/nucleus (Trx1 and Grx1) and the mitochondria (Trx2 and Grx2a). All these redoxins catalyze thiol-disulfide oxidoreductions via their active site cysteine (CXXC) residues and have a wide range of activities in redox biochemistry and redox signaling.The cytosolic/nuclear Trx1 and Grx1 are themselves regulated by oxidation and nitrosylation of structural Cys residues. In contrast the mitochondrial isoforms are generally unaffected by oxidation via reactive oxygen species or by NO donors like GSNO. The reversible oxidation of Trx1 by hydrogen peroxide generates monomeric two-disulfide molecules with C32-C35 and C62-C73 disulfides which are inactive as substrates for thioredoxin reductase (TrxR) but able to be reactivated by an autocatalytic process. The reversible oxidation od Trx1 is suggested to play a role in signaling by NADPH oxidases and the secretion of thioredoxin. Nitrosylation of C69 and C73 starting from fully reduced Trx1 also inactivates the molecule as a substrate for TrxR but is fully reversible. Nitrosylation of two-disulfide oxidized Trx1 generates C73 nitrosylated moleculers which can transnitrosylate and inactivate caspase 3. Since reduced Trx1 is a major denitrosylating agent for proteins it seems central to NO signaling. Nitrosylation or oxidation of the structural Cys residues in Grx1 may also reversibly control the catalytic activity involving glutathione-dependent oxidoreductions. The local and temporal inactivation of Trx1 and Grx1 by oxidation and nitrosylation is suggested to play a a role in signaling via thiol redox control of proteins with active site Cys residues like phosphotyrosine phosphatases.

Inactivation of glutathione peroxidase I in the red cells of Sickle cell patients and its elevation by hydroxyurea

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The auto-oxidation of hemoglobin to methemoglobin is a significant source of peroxide generation in red cells, and GPx1 is the most significant catalytic antioxidant in the red cell. GPx1 activity was measured in normal human red cells separated centrifugally in a discontinuous density gradient of Percoll. Activity but not amount of GPx1 decreases with red cell aging. By using nanoLC-ESI-q-TOF, GPx1 inactivation is shown to be associated with the conversion of the active site selenocysteine to dehydroalanine. In sickle cell, auto-oxidation of sickle cell hemoglobin is faster than normal adult hemoglobin and the resulting increased oxidative stress causes damages in erythrocyte. Hydroxyurea (HU), which is believed to induce the expression of γ-globulin, is widely used to treat sickle cell patients. We developed a microplate immunoaffinity capture assay for the measurement of the activity and the amount of GPx1 in cell extracts and applied to analyze GPx1 in HU- treated sickle cell patient (n=13), untreated sickle cell patient (n=9) and normal African-American (n=17). GPx1 activity is higher in sickle cell patients with HU treatment. HU induces Gpx1 expression through p53- and nitric oxide-dependent pathways in erythroblast cell line. The high GPx1 state is associated with reduced hemolysis, presumably due to reduced oxidative stress. These results suggest that increased de novo synthesis of Gpx1 by HU compensates for loss of Gpx1 activity by selenocysteine oxidation under severe oxidative stress and help protect red cells of anemia patient.

Glutaredoxin gene therapy in the diabetic heart

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A growing body of evidence supports the role of redox signaling in transmitting survival signals to the ischemic myocardium. Glutaredoxin, an important protein of thioredoxin superfamily has recently been found to play a role in cardioprotection. The present study was designed to examine if glutaredoxin-1 gene therapy could reduce cardiac dysfunction in the diabetic hearts.

Diabetes was induced by intravenous injection of streptozotocin. Male c57B1/J6 mice was randomized to intramyocardial injection of adenovirus encoding Grx-1 or empty vector. Seven days later, the hearts were subjected to 30 min of ischemia (I) and 2 h of reperfusion *ex vivo* and cardiac function were measured. Grx-1 expression was robust up to 14 days after gene transfer and was absent for empty vector. Grx-1 gene therapy provided cardioprotection to the diabetic hearts as evidenced by improved ventricular function and reduced infarct size and cardiomyocyte apoptosis.

A post-genomic view of phospholipid hydroperoxide glutathione peroxidase (GPx4)

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Twenty-five years after the discovery of GPx-4, the elucidation of a large number of genomes and structures permits a thorough phylogenetic analysis of this enzyme in the frame of the superfamily of GPxs and the disclosure of intriguing aspects about the alternative use of selenium or sulfur. Over 12 structures and more than 700 sequences analyzed, from Bacteria to Mammalia, the core structure and the catalytic site is highly conserved, while variability is limited to an oligomerization loop and a functional helix contributing to reducing substrate specificity. The oligomerization loop is missing in largest part of GPx, which are monomeric and contain a "resolving" Cys (CR) in the functional helix. This feature is associated to both the presence of a catalytic Cys (CP) at the active site and use thioredoxin as substrate. The emerging phylogenetic ancestor of the family is a monomeric GPx containing both a CP and a CR. These enzymes are therefore functionally overlapping Peroxiredoxins. Tetramerization is a relatively recent acquisition during evolution. The use of selenium is also a recent acquisition for both the largest part of tetrameric GPxs present only in Arthropoda, Vertebrata and Mammalia and for some monomeric GPxs (typically the GPx-4) spread throughout all kingdoms of life. Together with the introduction of the use of Sec at the active site, the functional helix containing the CR, and thus the use for Trx as reducing substrate is abrogated and the oxidized active site can now accept GSH as reductant. Remarkably, in mouse and rat the tetrameric GPx-5 and -6 are reverted back to a CP containing species. This set of information raises interesting evolutionary considerations regarding oligomerization, the use of active site selenocysteine, and the alternate use of Trx or GSH.

Glutathione peroxidases in cancer

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Hydroperoxides may contribute to cancer formation by initiating DNA lesions or by stimulating proliferation. By reducing hydroperoxides glutathione peroxidases (GPxs) would therefore be tumor-preventive. However, hydroperoxides initiate apoptosis and, thus, facilitate (tumor) cell removal. This implies that GPxs can also prevent elimination of tumor cells which would endow them pro-carcinogenic properties. Beyond, the ambiguous situation is complicated by the fact that individual GPxs have specific functions. Therefore the pro- or anti-carcinogenic properties have to be considered for each individual GPx. Chronic inflammation favours carcinogenesis. Cyclooxygenase-2 (COX-2) is generally up-regulated in both, inflammation and cancer. COXs and lipoxygenases (LOXs) require a certain hydroperoxide tone for activity and, accordingly, GPxs inhibit COX and LOX activities. Therefore the influence of GPx2 or GPx4 on COX2 expression as cancer-relevant process was investigated by genetic manipulation. Over-expressing of GPx4 suppressed eicosanoid production in L929 fibrosarcoma cells and drastically decreased tumor growth from these cells in nude mice (1). Knockdown of GPx2 by siRNA in HT29 colon cancer cells increased COX-2 expression and PGE2 production (2) indicating an anti-inflammatory role of GPx2. In contrast, the activation of the GPx2 promoter by the ß-catenin/TCF complex (3) which transfers Wnt signals points to a role of GPx2 in maintaining the intestinal homeostasis. Thus, an up-regulation of GPx2 might become a selective advantage for tumor cells. The findings demonstrate the complexity of individual functions of GPxs in physiological and pathophysiological conditions.

1. Heirman et al. FRBM 40, 2006;
2. Banning et al. ARS, 2008, in press;
3. Kipp et al. Biol. Chem. 388, 2007

SESSION V
LIPOIC ACID IN CELL SIGNALING AND TRANSCRIPTION

Regulation of the pyruvate dehydrogenase complex by lipoic acid

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R-Lipoic acid (R-LA) acts as a coenzyme in the oxidative decarboxylation of alpha-ketoacids by a family of the alpha-ketoacid dehydrogenase complexes in the mitochondria. One of these reactions involves the formation of acetyl-CoA from glucose-derived pyruvate by the action of the pyruvate dehydrogenase complex (PDC). R-LA also acts as an antioxidant and has been shown to increase glucose uptake and to lower serum lactate levels in diabetic subjects by activating insulin-signaling pathway. We have investigated the effect of R-LA on human PDC activity through its regulation by a mechanism involving phosphorylation. Four pyruvate dehydrogenase kinase (PDK) isoenzymes present in mammalian tissues regulate PDC activity by phosphorylation of the alpha subunit of the pyruvate dehydrogenase (E1) component. R-LA and S-LA had no significant effect on the activity of pyruvate dehydrogenase phosphatases. .Both R-LA and its reduced form dihydrolipoic acid (R-DHLA) caused inhibition of PDKs in the following order: PDK1 > PDK4 ~ PDK2 > PDK3. Interestingly, phosphorylation of sites 1, 2 and 3 of E1 by PDK1 was reduced to the same extent by R-LA. Since LA inhibited PDKs activities in the presence of E1 alone, dissociation of PDK from the lipoyl domains of the E2 component is not a likely explanation for observed inhibition. The reduction in autophosphorylation of PDK2 caused by R-LA indicated that lipoic compounds exerted their inhibitory effect on PDKs directly. It is suggested that LA may bind in a region of PDK interacting with the lipoyl domain of E2 and may affect its substrate binding ability. An inhibitory effect of R-LA on PDKs would result in less phosphorylation of E1 and hence an increase in PDC activity. This finding is consistent with the observed glucose and lactate lowering effects of R-LA in diabetic subjects.

Lipoic acid and lipoamide reduction by thioredoxin reductases

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For more than 10 years it has been known that lipoic acid and lipoamide are efficiently reduced by pure mammalian thioredoxin reductases using NADPH (Arnér et al BBRC (1996) 225: 268-274). However, it has not been clear to what extent that reaction is important in a cellular setting, i.e. for the reduction of lipoic acid or lipoamide added exogenously to cells. To address this question, we here studied A549 cells having the total thioredoxin reductase (TrxR) expression knocked down to about 10% compared to the level in control cells, using siRNA against TrxR1 encoded by the TXNRD1 gene. With these cells we subsequently assessed the total cellular lipoic acid and lipoamide reduction capacity. The results clearly showed that TrxR1 is the major NADPH-dependent reductase for lipoamide when analyzed using crude A549 cell protein extracts, with contribution of TrxR1 to more than 85% of that activity. Using instead an assay with living intact A549 cells, the reduction of lipoic acid or lipoamide was only impaired to about 50-70% upon TrxR1 knockdown. Those results suggest that within the intact cellular context, TrxR1 contributes to 30%-50% of the lipoic acid and lipoamide reduction capacity when these substrates are added exogenously to A549 cells. Thus, additional NADPH-dependent systems, such as glutathione-dependent enzymes, or NADH-dependent reducing systems, are likely to contribute to about 50%-70% of the total lipoic acid and lipoamide reduction in A549 cells. Alternatively, membrane-localized forms of TrxR1 that may be comparatively long-lived and furthermore are not analyzed using crude protein extracts, could be important for the lipoic acid/lipoamide reduction in a cellular context. On-going experiments are conducted to characterize these aspects in further detail.

1,2-Dithiole-3-thione induction of Nrf2 transcriptional networks

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Dithiolethiones such as 5-(2-pyrazinyl)-4-methyl-1,2-di-thiole-3-thione (oltipraz) and 1,2-dithiole-3-thione (D3T) are effective inhibitors of experimental carcinogenesis in vivo. D3T has been shown recently to be neuroprotective in a murine model of Parkinson's disease. These protective actions reflect enhanced expression of electrophile detoxication and antioxidative enzymes in target tissues, and are mediated in large part through activation of the Keap1-Nrf2 signaling pathway. The cancer chemopreventive efficacy of oltipraz and the neuroprotective efficacy of D3T are completely lost in Nrf2 transcription factor knockout mice. Gene expression patterns have been examined by oligonucleotide microarray analyses in vehicle- and dithiolethione-treated wild-type and nrf2-deficient mice to identify genes that may be critical to protection against environmental toxins. As expected, D3T increased the expression of genes through the Nrf2 pathway that directly contribute to the detoxication of toxins (e.g., glutathione transferases, UDP-glucuronosyl transferases) and that generate essential cofactors such as glutathione and reducing equivalents. Additional clusters included genes for chaperones, protein trafficking, ubiquitin/26S proteasome subunits, and signaling molecules. Combined genomic and proteomic comparisons of global expression patterns between wild-type and nrf2-deficient fibroblasts revealed very similar patterns and surprisingly concordant modulation of transcript and protein levels. Thus, the Nrf2 pathway regulates the expression of genes that enhance cell survival and serves as an attractive target for chemopreventive agents that may prevent or delay the onset of chronic diseases.

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Age-related loss of Nrf2-mediated gene transcription: Improvement by lipoic acid

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The elderly are known to be at heightened risk for a variety of acute and chronic pathologies, in part due to a decline in certain cellular stress defense mechanisms. This lack of an adequate stress response elevates the risk for oxidative and toxicological insults. Thus, a practical approach to improving elder health would be to maintain or reverse the loss in stress resistance mechanisms. We observed that feeding old rats (R)-α-lipoic acid (R-LA) significantly increased hepatic antioxidant levels and markedly improved resistance to toxins. R-LA did not achieve this by merely acting as a dietary antioxidant; rather, it reversed the age-related loss of endogenous antioxidant capacity, especially glutathione (GSH). This led us to the discovery that nuclear levels of NF-E2 related-factor 2 (Nrf2), a transcription factor that regulates expression of GSH-synthesizing enzymes, markedly declines with age but LA reverses this loss. Our work is now focused on understanding why Nrf2-mediated stress defenses decline with age and the precise mechanism(s) how R-LA acts to maintain this vital cellular defense system. Our focus is two-fold, namely to define age-related dysregulation of signaling pathways that may adversely affect Nrf2 nuclear translocation and tenure as well as determine the extent that the aging process alters the Nrf2 transcriptome at the gene level. Results indicate that a bimodal problem arises with age: nuclear Nrf2 levels significantly decline and a repressive transcriptional motif also develops that together, limits ARE-mediated gene expression. R-LA improves Nrf2 action by increasing nuclear tenure of Nrf2 and also promoting its binding to an imperfect, alternative ARE sequence. Thus, R-LA may be part of a unique class of dietary micronutrients that promote “healthspan” by maintaining vital cellular defenses, which otherwise decline with age.

α-Lipoic acid prevents lipotoxic cardiomyopathy in acyl-CoA-synthase transgenic mice

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α-Lipoic acid (α-LA) mimics the hypothalamic actions of leptin on food intake, energy expenditure, and activation of AMP-activated protein kinase (AMPK). To determine if, like leptin, α-LA protects against cardiac lipotoxicity, α-LA was fed to transgenic mice with cardiomyocyte-specific overexpression of the acyl CoA synthase (ACS) gene. Untreated ACS-transgenic mice died prematurely with increased triacylglycerol content and dilated cardiomyopathy, impaired systolic function and myofiber disorganization, apoptosis, and interstitial fibrosis on microscopy. In α-LA-treated ACS-transgenic mice heart size, echocardiogram and TG content were normal. Plasma TG fell 50%, hepatic-activated phospho-AMPK rose 6-fold, sterol regulatory element-binding protein-1c declined 50%, and peroxisome proliferator-activated receptor-gamma cofactor-1α mRNA rose 4-fold. Since food restriction did not prevent lipotoxicity, we conclude that α-LA treatment, like hyperleptinemia, protects the heart of ACS-transgenic mice from lipotoxicity.

A comparison of the pharmacokinetics profiles of lipoate enantiomers with the racemic mixture in healthy human subjects indicates possible stereoselective gut transport

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Lipoic acid (LA) exists as two enantiomers, the naturally occurring R-lipoic acid (RLA) and S-Lipoic acid (SLA) but is generally administered as a racemic mixture (rac-LA) for therapeutic and supplemental uses. The PK profiles of rac-LA and RLA are known but no human data has been reported for the single unnatural enantiomer, SLA. To test whether SLA positively or negatively alters gastrointestinal absorption and subsequent PK, 600 mg of stabilized sodium salts of RLA, SLA and racemic-LA (rac-LA) were administered (p.o. dissolved in 200 mL water) in a classical three-period cross-over study undertaken with three healthy human subjects. METHODS: Total plasma RLA, SLA and rac-LA content were determined by the percent recovery using high-performance liquid chromatography [electrochemical/coulometric detection; HPLC/ECD]. Results: The mean values for Cmax and AUC show that RLA values are considerably greater than either rac-LA or SLA (RLA Cmax =15.67 µg/mL [78 µM], RLA AUC=6.86 µg hr/mL; SLA Cmax = 6.43 µg/mL, SLA AUC= 3.89 µg hr/mL; rac-LA Cmax = 6.37 µg/mL; rac-LA AUC=2.69 µg hr/mL. The time to maximum concentration (T max) reveals SLA>RLA>rac-LA (18.33, 13.33, 10.00 minutes) and the elimination half life (T 1/ 2) SLA>rac-LA>RLA (28.87, 20.7, 12.5 minutes). Conclusion: The human Cmax and AUC values of RLA are significantly greater than either rac-LA or SLA when administered as oral solutions of the sodium salts. The fast T 1/ 2 and T max values of RLA indicates a possible stereoselective transport mechanism, benefited when SLA is absent. Both RLA and SLA, as the single enantiomers are absorbed more rapidly and to a greater extent, resulting in higher Cmax and PK values relative to enantiomeric components of the racemic mixture, indicating competition for the transporter.

SESSION VI
CLINICAL STUDIES ON LIPOIC ACID

Anti-inflammatory therapeutic direction by thioredoxin family proteins

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Human thioredoxin (TRX) is a 12 kDa multifunctional redox protein. TRX suppresses inflammatory responses by regulating neutrophil activation and extravasation. TRX overexpression mice were resistant to systemic inflammation against cigarette smoking and colitis through the suppression of Macrophage migration inhibitory factor (MIF) expression in mouse models. Previously, we have shown that MIF was co-purified with TRX from ATL-2 cells and reported that the expressions of TRX and MIF were reciprocally regulated. We observed that TRX bound to MIF in intra-, or extracellular compartments of ATL-2 cells and that TRX inhibited the internalization of MIF into ATL-2 cells. Furthermore, rTRX inhibited rMIF-mediated enhancement of TNF-alpha production from macrophage RAW264.7 cells. These findings suggest that TRX interferes the internalization of MIF into target cells by binding to MIF and thereby regulates its pro-inflammatory activity. Accumulating evidence suggests the anti-inflammatory effect of TRX as well as its anti-oxidative and anti-apoptotic properties is beneficial for treatment of various lung disorders such as ARDS/ALI, interstitial pneumonia, and COPD. Either overexpression or injection of TRX ameliorated bleomycin- or LPS-induced acute lung injury in rats, IL2/IL18-induced interstitial pneumonia in mice, and cigarette smoke-induced pulmonary emphysema in mice. Based on these findings, clinical trials of intravenous TRX administration against ARDS/ALI are now under preparation in our institution. We propose a novel anti-inflammatory activity of TRX in which TRX not only inhibits neutrophil activation but also regulates MIF-mediated inflammatory response.

α-Lipoic acid improves symptomatic diabetic polyneuropathy: Preclinical and clinical evaluations

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Chronic hyperglycemia can be induced in experimental diabetic neuropathy (EDN). We have demonstrated physiologic abnormalities of impaired endoneurial perfusion and hypoxia, and duplicate nerve conduction slowing seen in human diabetic neuropathy. Indices of oxidative stress and injury include reduced GSH, increased superoxide anion and lipid peroxidation. Manifestations of oxidative damage to nerve fibers and Schwann cell have included caspase-3 and 8-hydroxydeoxyguanosine and TUNEL positivity, and in chronic state results in nerve pathological changes. These changes have been dose-dependently reversible following treatment with α-lipoic acid (ALA). Human studies are summarized in a meta-analysis comprising 1,258 diabetic patients with symptomatic diabetic neuropathy from four randomized clinical trials, and we found a significant improvement in symptoms accompanied by an improvement of neuropathic deficits. In a subsequent four-arm, randomized, double-blind, placebo-controlled, dose response trial using ALA (600, 1,200, and 1,800 mg q.d.), patients received treatment over 5 weeks after a 1-week placebo run-in period. Mean total symptom score (TSS) did not differ significantly at baseline among the treatment groups and on average decreased by 4.9 points (51%) in ALA600, 4.5 (48%) in ALA1200, and 4.7 (52%) in ALA1800 compared with 2.9 points (32%) in the placebo group (all $P < 0.05$ vs. placebo). The corresponding response rates (≥50% reduction in TSS) were 62, 50, 56, and 26%, respectively. Significant improvements favoring all three ALA groups were also noted for stabbing and burning pain, the NSC score, and the patients' global assessment of efficacy.

Effect of α-lipoic acid on experimental diabetic neuropathy

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Hyperglycemia-induced mitochondrial overproduction of reactive oxygen species (ROS) is central to the pathogenesis of late diabetic complications including diabetic neuropathy (DN). In this context, various experimental animal models have shown that activation of the redox-sensitive transcription factor NF-κB and subsequent expression of NF-κB regulated gene products contributes to both, pain and loss of pain perception. Ligation of advanced glycation end products (AGEs) to their surface receptor RAGE is central in regulating NF-κB due to its capability to perpetuate NF-κB activation. Experimental animal models have recently provided evidence that reduced detoxification of AGEs by the glyoxalase-system, engagement of the receptor RAGE and RAGE dependent sustained activation of NF-κB significantly contribute to functional deficits in diabetic neuropathy. Consistently, early pain and the subsequent reduction in neuronal function are blunted in diabetic $RAGE^{-/-}$-mice. α-Lipoic acid, which has advantages over classical antioxidants as it distributes to the mitochondria, is regenerated by glycolytic flux and recycles cellular antioxidant redox-pairs due to its low redox potential, reduces AGE-formation and accumulation, AGE-induced ROS–formation, RAGE expression, NF-κB p65 antigen and NF-κB binding *in vitro* and i*n vivo*. Furthermore, loss of pain perception indicative for long-standing neuropathy is reversed in diabetic mice upon treatment with a-lipoic acid. Thus, part of the beneficial effect of a-lipoic acid in diabetic neuropathy includes the disruption of the AGE-RAGE- NF-κB axis.

POSTERS

Effect of chronic alcohol consumption on rat brain mitochondria

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Introduction: Glutathione (GSH) plays an important role in the integrity of mitochondria and, the regulation of mitochondrial GSH (mGSH) depends on appropriate membrane dynamics, which in turn, is affected by cholesterol deposition on mitochondrial membranes. In liver from alcoholic rats, cholesterol deposition in mitochondria leads to severe mGSH depletion and the release of proapoptotic factors such as cytochrome c. Since alcohol exposure can also cause serious damage on CNS, the aim of this study was to demonstrate the existence of similar mechanisms in the brain of alcoholic rats. Material and methods: Animals received either control or an alcohol liquid diet (Lieber-De Carli) during 6 weeks. After sacrifice brains were homogenized and fractionated to obtain mitochondrial fractions. GSH in whole brain homogenate, mGSH and mitochondrial cholesterol were measured by HPLC. It was also determined the release of cytochrome c by western blot after apoptotic stimuli (Atractyloside). Results: Brain GSH levels were not affected by alcohol consumption; however mGSH was decrease in the brain of alcoholic rats. This decrease was accompanied by a higher deposition of cholesterol in mitochondrial membranes, and by an increase in the release of cytochrome c after stimulating mitochondria with atractyloside. Conclusion: Although brain GSH levels are not affected in alcoholic rats, mitochondrial uptake of GSH is impair, possibly due to the increase in cholesterol deposition, similar to what happens in alcoholic liver. These alterations sensitize mitochondria to apoptotic stimuli allowing the release of cytochrome c, which in turn can induce caspase-dependent cell death.

Antioxidants are useful to prevent oxidative stress alterations in CNS and hippocampal apoptosis in experimental diabetes

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Background/aims: Diabetes mellitus increases the risk of central nervous system disorders. The mechanisms responsible for the increased risk of these disorders are incompletely understood. One of the mechanisms by which hyperglycemia causes neural degeneration is via the increased oxidative stress that accompanies diabetes. Methods: Wistar male rats were made diabetic with streptozotocin and were studied over 3 months after onset of diabetes. Hippocampus and cortex homogenate was used to assay malondialdehyde (MDA) and GSH content and GSH peroxidase (GPx) activity. Hippocampi were processed for detection of apoptotic cells by TUNEL analysis and activated caspase-3. As a functional test of cognition the Morris water maze test was assayed. The number of BrdU-positive cells were also counted, as an indicator of cell proliferation. Results: Streptozotocin-induced experimental diabetes in rats promoted oxidative stress in hippocampus and cortex after 3 months of hyperglycaemia. Lutein and docosahexaenoic acid (DHA) administration prevented the alterations of oxidative stress markers (MDA concentration, GSH content and GPx activity). Morris test showed prolonged latencies associated with the number of TUNEL-positive nuclei and caspase-3 positive cells that were increased significantly in hippocampus after 3 months of diabetes and both antioxidants normalized the modifications in hippocampus mentioned above. Streptozotocin-induced diabetes produce a dramatic decrease in cell proliferation in the dentate gyrus prevented in the groups treated with antioxidants. Conclusions: These studies demonstrate that cells from CNS die by apoptosis in diabetes and antioxidants can be an adjuvant therapy to prevent this death.

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Antioxidant effect of lutein and docosahexaenoic acid on lipid peroxidation and diabetic retinal function

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Background/aims: Diabetic retinopathy is the major cause of adult blindness in developed countries. However, it is still not known the pathogenic link between hyperglycaemia and diabetic complications. It has been repeatdly suggested that oxidative stress may play a role in the process and antioxidants can be useful as a supportive therapy in diabetes. We have studied lipid peroxidation on diabetic rat retina and the neuroprotective effects of the antioxidant lutein and docosahexaenoic acid (DHA). Methods: Wistar male rats were made diabetic with streptozotocin and were studied over three months. Immediately before death electroretinograms were recorded. One retina was fixed, cryoprotected and cryosectioned for detection of apoptotic cells by TUNEL analysis and activated caspase-3 and the other retina was homogenized in prechilled 0.2 M potassium phosphate buffer. This homogenate was used to assay MDA, GSH content and GPx activity. Results: After 3 months of diabetes, antioxidant administration prevented not only the alterations of oxidative stress markers (tissue GSH and malondialdehyde (MDA) concentration and GPx activity) but also the impairment of retinal function (as assessed by the modifications in electroretinogram latency time and b wave amplitude). The number of TUNEL-positive nuclei and caspase-3 positive cells increased significantly in retina and both antioxidants normalized the modifications. Conclusions: The data suggest that lutein and DHA are useful in preventing diabetic retina complications.

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The effect of *Ginkgo biloba* extract on the amyloid-precursor-protein processing

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The *Ginkgo biloba* extract EGb761 is widely used to prevent cognitive disorders including Alzheimer disease (AD). Even though neuroprotective properties of EGb761 are consistently reported the molecular mechanisms and the specific role of the major constituents of EGb761 (flavonols and terpenlactones) are largely unknown. One major hallmark of AD is the deposition of b-amyloid (Ab) as amyloid plaques. Ab is a result of amyloid precursor protein (APP) cleavage catalysed by b-secretases (BACE). Alternatively, APP can be processed by a -secretases (ADAM) which lead to a soluble non pathogenic product. The aim of the present study was to investigate the efficacy of EGb761 and its flavonol and terpenelacton fractions to modulate BACE-1 enzyme activity and mRNA level in vitro, in Neuro-2a cells treated with EGb761 and in brain tissues of C57B6 mice fed a diet enriched with EGb761 or its flavonol or terpenelacton fraction for 4 weeks. Additionally, BACE-1 gene expression and enzyme activity in a mouse model for AD, the transgenic Tg2576 mice, was investigated after feeding them EGb761 for 16 months. In both mice strains the ADAM and APP gene expression was investigated. Neither EGb761 nor its fractions affected BACE-1 activity in vitro. Furthermore, in Neuro-2a cells and C57B6 as well as Tg2576 mice no effect on BACE-1 enzyme activity and mRNA levels was observed. Additionally, in both mice strains neither APP nor ADAM mRNA levels were affected by EGb761 treatment. Current findings suggest that BACE, ADAM and APP may not be major molecular targets of EGb761 or its main fractions.

Genome-wide response of human aortic endothelium cell to triglyceride-rich lipoprotein ;ipolysis products

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The endothelium plays an important role in the control of the vascular system. Triglyceride rich lipoproteins (TGRL) are an independent risk factor for coronary artery disease and have been linked to atherosclerosis. We hypothesized that TGRL lipolysis products contribute to pathogenesis of atherosclerosis by affecting the expression of multiple pro-inflammatory and pro-coagulant genes. To test this hypothesis, we incubated human aortic endothelial cell (HAEC) with either TGRL or TGRL lipolysis products for 3 hrs. TGRL were isolated from postprandial human plasma and incubated with lipoprotein lipase and the resulting lipolysis products were added to HAEC in culture. Total RNA was extracted from these cells and the TGRL or TGRL lipolysis products sensitive transcriptomes were obtained using high density oligonucleotide arrays (U133A 2.0 array). 266 of 285 genes were up-regulated and 19 genes were down-regulated by TGRL lipolysis products in comparison to TGRL alone. Functional classification of the affected genes identified transcription factors, cytokines, growth factors, cell cycle and inflammation-related genes. Specifically, TGRL lipolysis products up-regulated adhesion molecules (E-selectin and VCAM-1); cytokines (IL6, IL1α) and activating transcription factor 3 gene expressions. Expression of these genes was confirmed by quantitative real-time RT-PCR. Our results indicate that TGRL lipolysis initiate a stress response in HAEC that has the molecular signature of inflammation. These preliminary data from in vitro challenged of HAEC support our hypothesis showing that TGRL lipolysis products modulate multiple pro-inflammatory genes simultaneously that may contribute to the pathogenesis of atherosclerosis.

Short-term diabetes reduces the NADPH-diaphorase reactivity in mice striatum

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The NADPH diaphorase (NADPH-d) histochemistry has been used as marker for nitric oxide synthase (NOs) neuronal populations. Indeed, NADPH-d histochemistry is the result of the NADPH-reductase (NADPH-r) activity under certain conditions. This NADPH-r takes part of the homodimeric conformation of all the three isoforms of NOs and its activity is necessary for the right enzymatic functionality. Nitric oxide not only acts as a modulator under normal physiologic condition but also under pathological conditions can play other roles. Diabetes mellitus is a common pathology that can affect the redox potential of the cell and thereafter several tissues. It has been previously reported a reduction of the neuronal nitric oxide synthase activity in the cerebellum. A reduction of the NADPH-d activity has also been reported in the retina. However, an increase of the density of NADPH-d positive neurons has been shown in the hypothalamus of diabetic rats. Herein we report that a short time (15 days) of uncontrolled diabetes leads to a significant reduction of the NADPH-d neuron density in the striatum without affecting the nNOs neuronal density. Pathophysiological implications are discussed.

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A novel source of reactive oxygen species in the mitochondria

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Oxidative stress has been implicated in diverse human diseases including diabetes. The bulk of oxidative pathways are harbored in the mitochondria, where various redox carriers leak electrons to oxygen to form superoxide anion. In phagocytic cells, however, gp91phox-based NAD(P)H oxidase has long been recognized as a major source of reactive oxygen species (ROS). The gp91phox isoform Nox4 was cloned from the kidney and we have recently shown that it is a major source of ROS in renal cells and kidney tissue of diabetic animals. We generated specific rabbit polyclonal Nox4 antibodies and found that Nox4 localizes to mitochondria. Several approaches were utilized to confirm Nox4 localization. (i) Immunoblot analysis in cultured rat glomerular mesangial cells (MCs) as well as renal cortex revealed that Nox4 was present in crude mitochondrial fractions, in mitochondria-enriched heavy fractions and in purified mitochondria. (ii) Immunofluorescence confocal microscopy was also used to localize Nox4 in MCs. Our observation was confirmed using the mitochondrial localization prediction program MitoProt, where the probability score for Nox4 obtained was identical to mitochondrial protein human cytochrome c oxidase subunit IV. Functionally, siRNA-mediated knockdown of Nox4 reduces NADPH oxidase activity in pure mitochondria and blocks glucose-induced mitochondrial superoxide generation. Our data provide the first evidence that a functional Nox4 is present and regulated in mitochondria, indicating the existence of a novel source of ROS in this organelle. The present demonstration that Nox4 resides in mitochondria and plays a key role in disease-induced oxidative stress may reconcile the mitochondrial and the NAD(P)H oxidase hypotheses.

Brain mitochondrial dysfunction in aging

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Aging of mammalian brain is associated with a continuous decrease of the capacity to produce ATP by oxidative phosphorylation. The impairment of mitochondrial function is mainly due to diminished electron transfer by complexes I and IV, whereas inner membrane H^+ impermeability and F_1-ATP synthase activity are only slightly affected. Dysfunctional mitochondria in aged rodents show decreased rates of respiration and of electron transfer, decreased membrane potential, increased content of the oxidation products of phospholipids and proteins and increased size and fragility. In aging mice, the activities of brain mitochondrial enzymes (complexes I and IV and mtNOS) are linearly correlated with neurological performance (tightrope and T-maze tests) and with median life span and negatively correlated with the mitochondrial content of lipid and protein oxidation products. Conditions that increased mice median life span, such as moderate exercise, vitamin E supplementation, caloric restriction, and high spontaneous neurological activity, also improved neurological performance and mitochondrial function in aged brain. The diffusion of mitochondrial NO and H_2O_2 to the cytosol is decreased in the aged brain and may be a factor for reduced mitochondrial biogenesis.

Cardiac mitochondrial nitric oxide: Hemorrhagic shock in conscious and anesthetized rats

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Hypovolemic state induced by acute hemorrhage triggered a heterogeneous and dynamic nitric oxide synthases (NOS) activation. An increased cardiac endothelial NOS expression is an early molecular response to regulate heart rate after blood loss. The inducible NOS become a major source of cardiac NO production in the later stages, which could be determinant of the heart dysfunction after 120 min of sustained hemorrhagic shock. Our aim was to evaluate the involvement of mitochondrial NOS (mtNOS) activity in the cardiovascular adaptation to hemorrhagic shockc after 120 min of bleeding in conscious and anesthetized rats. Groups of animals (n=5 per group): C: normotensive conscious and anesthetized rats; CH: conscious hemorrhaged rats (20% blood loss); AH: anesthetized hemorrhage rats. No differences in the succinate-supported state 4 and 3 respiration (ng-at O/min.mg protein) were observed among the experimental groups ($C_{state\ 4}$ = 130 ± 9.8, $C_{state\ 3}$ = 234 ± 16; $CH_{state\ 4}$ = 112 ± 10, $CH_{state\ 3}$ = 204 ± 15; $AH_{state\ 4}$ = 158 ± 11, $AH_{state\ 3}$ = 240 ± 11). Bleeding did not modify heart mitochondrial hydrogen peroxide production (nmol H_2O_2/min.mg protein) compared with C (C = 0.577 ± 0.05; CH = 0.569 ± 0.1; AH = 0.524 ± 0.06). However, the hypovolemic state modified heart mtNOS functional activity determined by enhancing H_2O_2 production (C = 59%; CH = 85%; AH = 67%). Taking into account that changes in mtNOS functional activity reflect variations in mitochondrial NO production and steady state concentration, higher mitochondrial NO levels would be involved in the cardiovascular adaptation to volume depletion observed in conscious animals.

Plasma pharmacokinetics of R-(+)-lipoic acid administered as sodium R-(+)-lipoate to healthy human subjects

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Rationale: Lipoic acid (LA) has a single chiral center (at C6) and exists as two enantiomers, the naturally occurring R-lipoic acid (RLA) and S-Lipoic acid (SLA) but is widely administered as a racemic mixture (rac-LA. The alkali metal salts (K^+ and Na^+) of rac-LA have been reported and used successfully in various animal models of aging, where they were able to reverse markers of aging to match those of young animals. A pharmacokinetic (PK) trial using the sodium salt of RLA (NaRLA) has not been reported and it is unknown if plasma concentrations achieved in animals can be achieved in humans. Study Design: A PK trial using 12 healthy human subjects was undertaken. Subjects consumed 600 mg RLA (as NaRLA, based on the RLA content) dissolved in 200 mL purified water. In addition to the one 600-mg dose, subject 3 received three 600-mg doses RLA (as NaRLA) at t = 0, 15, and 30 minutes, in order to determine the effect of three doses on Cmax and AUC relative to a single dose in the same subject. Methods: Whole blood was drawn, plasma separated and the RLA content analyzed by HPLC/ECD. Results: The average dose was 8.25 mg/kg, generating a mean C_{max} of 16.03 mcg/mL (range: 10.6-33.8 mcg/mL), median T_{max}=15 minutes (range: 10-20 minutes), and mean AUC of 441.59 mcg min/mL (7.36 mcg hr/mL). Subject 3 consumed three 600-mg doses of RLA (as NaRLA) resulting in a C_{max} of 21.9 mcg/mL, AUC of 1,049 mcg min/mL (17.48 mcg x hr/mL), and extended the T_{max} out to 45 minutes.

Reduced mitochondrial H_2O_2 results in characteristics associated with longevity in transgenic mice overexpressing peroxiredoxin 3

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H_2O_2 is one of the major reactive oxygen species produced by mitochondria, and data from mammalian animal models indicated that mitochondrial H_2O_2 plays a key role in aging. The reason why mitochondrial H_2O_2 is important in aging may be due to H_2O_2 role not only in inducing oxidative damage but also in regulating cell signaling. Peroxiredoxin 3 (Prdx3/Prx3) is a thioredoxin peroxidase specializing in scavenging H_2O_2 in mitochondria. To validate the anti-aging effect of reduced mitochondrial H_2O_2, we generated a novel transgenic mouse model overexpressing Prdx3: Tg(PRDX3) mice. Tg(PRDX3) mice were generated using a human endogenous PRDX3 gene. Compared to control Wt mice, Tg(PRDX3) mice have a 3- to 5- fold increase in both Prdx3 mRNA and Prdx3 protein in all tissues. Co-localization study indicates that all of the expressed Prdx3 protein is localized within mitochondria in Tg(PRDX3) mice. Isolated mitochondria from brain and muscle tissues of Tg(PRDX3) mice produced significantly reduced amount of H_2O_2, as determined using a Amplex red HRP method. Tg(PRDX3) mice had reduced oxidative damage (measured as F2-isoprostanes level) and altered cell signaling (e.g., reduced mTOR signaling). Fibroblasts from Tg(PRDX3) mice showed increased resistance to oxidative stress induced cell death and apoptosis. In addition, Tg(PRDX3) mice showed improved glucose homeostasis, as evidenced by reduced plasma glucose level and improved glucose tolerant test. Thus, reduced mitochondrial H_2O_2 in Tg(PRDX3) mice resulted in characteristics associated with longevity, suggesting that reduction in H_2O_2 production in mitochondria could serve as anti-aging mechanism.

Ascorbate as a pro-oxidant agent decreases tumor growth *in vivo*

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We have revisited the question of efficacy for high dose ascorbate therapy in cancer. In vitro cytoxicity of select cancer cells was observed to H_2O_2 generated by exposure to ascorbate (34/43 cell types: IC_{50} < 10mM). Normal cells were resistant (5 cell types: IC_{50} >20 mM). In animal models, we demonstrated tight control of ascorbate absorption through the gut limits uptake to < 0.2 mM regardless of oral dose. Pharmacologic ascorbate concentrations achieved via i.v. or i.p. injection, have pro-oxidant actions by generating ascorbate radical and H_2O_2. Ascorbate *in vivo* efficacy was tested using murine models of glioblastoma, pancreatic carcinoma and ovarian carcinoma. Despite differences in cancer type, i.p. ascorbate as the only treatment significantly decreased tumor growth and tumor weight 40 to 50% (P = 0.04 to 0.001). Metastases, present in approximately 30% of 9L glioblastoma controls, were absent in ascorbate treated animals. The generation of ascorbate radical and H_2O_2 within tumor and the subcu sites were quantified by EPR and fluorescence. A single 4g/kg i.p. dose increased ascorbate in both blood and tissue > 600 fold (peaks at ~30 mM). Ascorbate radical in both tumor and subcu extracellular fluid increased from < 10 nM to > 500 nM, with blood levels < 50 nM. H_2O_2 was preferential generated within tumors, increasing from basal levels of 25 µM to a plateau of 150 µM. In human subjects who received escalating doses of i.v. ascorbate, peak plasma concentrations were 30 mM, similar to those concentrations in mice given i.p. ascorbate. These data indicate that H_2O_2 is a pro-oxidant species mediating ascorbate therapeutic effects in murine models of cancer. High dose of parenteral ascorbate with clinical relevance were beneficial in treating several tumor types known for their aggressive growth and limited treatment options.

Modulation of age-related vascular inflammation by calorie restriction and dietary betaine

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Vascular aging can be considered as the fundamental process of the organism¡¯s aging. Our present study was launched to further elucidate the molecular aspects of aortic pro-inflammatory genes regulation during aging by utilizing two experimental paradigms; anti-inflammatory calorie restriction (CR) and dietary supplementation of phytochemical betaine with various amounts on specific pathogen free Fischer 344 rats. Result showed that reactive species (RS) and lipid peroxidation products 4-HNE/MDA levels were all increased, accompanied with decreased total thiol contents. In addition, the aortic anti-oxidative capacity was also found to be decreased in old rats. The anti-inflammatory CR and dietary betaine effectively suppressed these oxidative stress related markers. To further look into the molecular event involved in pro-inflammatory gene activation in aging aorta, levels of various inflammation biomarkers were determined, including PGE2, TXA2, COX-2, PGES, PGIS, P-/E-selectin, VCAM-1 and ICAM-1, as well as TNF-α and IL-1β. Results showed that those genes were all increased with age, and importantly, they were attenuated by CR and betaine supplementation. Based on these finding and others, we concluded that the protection of aging vasculature by anti-aging action of CR is mediated through its anti-inflammatory property. Similarly, the beneficial action of dietary betaine on vascular functions is likely related to its anti-oxidative and anti-inflammatory actions. [KOSEF-2007-00376]

Inflammatory process as the underlying mechanism for ionizing radiation

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The biological effects of ionizing radiation are well-known for the cause of oxidative damage and cellular redox imbalance. In the present study, we investigated the molecular mechanisms underlying irradiation-induced alteration of signal pathways by closely examining a redox-sensitive transcription factor, NF-κB and various pro-inflammatory gene expressions. The experimental animals were C57/BL6 mice that were exposed to various doses of g- and X-rays, and oxidatively vulnerable kidney tissues were used. Our results clearly showed that a short-term irradiation can readily alter signal pathways causing NF-κB activation. Furthermore, we obtained molecular evidence showing the up-regulation of several major NF-κB-dependent pro-inflammatory genes such as COX-2, iNOS, VCAM1, ICAM1, and E-selectin. We also found that ionizing radiation caused oxidative stress to these animals which led to a dose-dependent redox imbalance as shown by key redox markers. Our findings on phosphorylated ERK, JNK, and p38 MAPKs further revealed molecular insights into the pro-inflammatory activation of ionizing radiation. The significance of the current study is the new molecular information on the up-regulation of various pro-inflammatory genes through NF-κB activation via the MAPKs pathway. Considering molecular evidence on the exquisitely sensitive NF-κB to ionizing irradiation, the activated various pro-inflammatory mediators, and the altered redox status, we propose that the major biological damaging effect of ionizing irradiation may be the subtle activation of inflammatory processes through the disruption of redox-sensitive cellular signaling pathways. [KOSEF-2007-00376]

Attenuation of age-related inflammation by Zingerone treatment

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Zingerone, a major component found in ginger root, has been known as anti-mutagenic and anti-carcinogenic activities that are often associated with its anti-oxidative and anti-inflammatory activities. In recent studies, we examined molecular mechanism of zingerone treatment on proinflammatory NF-κB activation via the redox-related NIK/IKK and MAPK pathways. The results showed that zingerone had not only the anti-oxidant effect by constitutive suppression of RS, but also anti-inflammatory effects by suppression of NF-κB activation in aged rat. In addition, zingerone treatment suppressed gene activation of proinflammatory enzymes, COX-2 and iNOS, which were upregulated with aging through NK-κB activation and IKK/MAPK signaling pathway. These experiments strongly indicate that zingerone treatment exerts an beneficial efficacy by suppressing both oxidative stress and age-related inflammation through the modulation of several key pro-inflammatory genes and transcription factors. Thus, the significance of our findings is that the zingerone treatment may provide some preventive measure against chronic inflammatory conditions that underlie many age-related inflammatory diseases, such as metabolic syndrome, cardiovascular disease, dementia, arthritis, diabetes, osteoprosis and cancers.

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Interactions of large procyanidins with membranes and the regulation of cell signaling

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We have presented evidence that large procyanidins (LP) can interact with membranes and protect intestinal cells from different pro-inflammatory stimuli. We have now tested if LP could prevent the triggering of different events that has been associated to lipid rafts, i.e calcium mobilization, oxidant formation, and activation of select cell signals. To test this hypothesis, we used liposomes, and Caco-2 cells as a model of intestinal epithelium, and investigated the effects of a fraction enriched in LP isolated from cocoa. LP prevented Triton-X 100-mediated disruption of synthetic liposomes enriched with glycoplipids (main components of lipid rafts). In Caco-2 cells, LP inhibited NF-κB activation initiated by different pro-stimulatory compounds, showing the highest inhibitory capacity for signaling initiated at lipid rafts (e.g. tumor necrosis factor alpha). Furthermore, LP inhibited deoxycholate-induced calcium mobilization, oxidant production, and activation of mitogen-activated kinases (MAPKs). In summary, LP can interact with synthetic and biological membranes and protect them from different pro-inflammatory stimuli. The obtained results suggest that LP could have a relative selectivity to interact with particular areas of the membrane, e.g. lipid rafts, and to modulate oxidant and signaling events initiated in these areas.

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Humanin isoforms are differentially regulated by β-carotene in HUVECs

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Background: Humanins (HNs) are a group of distinct endogenous peptides, encoded within the mitochondrial and nuclear genomes, with a broad spectrum of cytoprotective and antiapoptotic properties mediated by the interactions with the members of the Bax/Bcl-2 pathway. We have recently shown that β-carotene (BC) prevents apoptosis in human umbilical vein endothelial cells (HUVECs) through the modulation of the same pathway. Aims: To investigate if HN isoform expression is regulated by BC in HUVECs. Methods: Expression rates of HN genes were compared quantitatively (qRT-PCR) in HUVECs exposed to 3 μM BC for 6 and 24 h. Results: Following a six-hour incubation with BC all of the HN genes became downregulated (2-8-fold) except for HN5, whose expression increased eight-fold. At 24 hours, HNM (mitochondrial izoform), HN10b, and HN16 remained downregulated, while HN5 decreased to baseline. The expression of the remaining genes increased, either returning to baseline (HN10a, HN6, HN11, HN7), or rising more than eight-fold above baseline (HN20, HNX). Conclusions: In HUVECs, HN izoforms are differentially regulated by physiologically detected blood levels of BC in a time-dependent fashion. The interaction between HN and BC is likely to involve the common Bax/Bcl-2 pathway of apoptosis regulation.

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The effect of the LC n-3 PUFA dietary intervention on the proatherogenic LDL phenotype and ischemia-modified albumin (IMA) related to postprandial response. The LIPGENE study.

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LDL phenotype B i.e. the predominance of highly oxidizable small particles, has been highlighted as a risk factor for CHD. During the postprandial state, there is an increase in circulating triacylglycerol fractions, accumulating in atheromatous plaques. Increased blood level of ischemia modified albumin (IMA) reflects episodes of muscle ischemia and oxidative stress. EU F6 LIPGENE Human Dietary Intervention Study patients with metabolic syndrome subgroup (n=99) received isocaloric diets: (A) High-fat SFA-rich or (B) MUFA-rich; (C) low-fat, high-complex carbohydrate diet + placebo or (D) 1.24 g/d LC n-3 PUFA for 12 weeks. Pre- and post-intervention postprandial test with same fat composition as on assigned diet was done. LC n-3 PUFA (D) and MUFA-rich diet (B) resulted in favorable alteration of LDL phenotype (from B to A), diminished oxidative stress and improved antioxidant properties. Postprandial state was connected with the increased LDL particle density and IMA content. IMA value differentiated responders and non-responders to diet D. The ratios of plasma n-3/n-6PUFAs (% of mass), apoCIII/apoCII and TRLcho l/apoE were predictors of the post-dietary LDL phenotype B to A transformation. Thus dietary intervention may modify proatherogenic risk factors in course of metabolic syndrome with NO effect on plasma lipid concentrations.

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Roles of iNOS, nNOS, and protein nitration in peripheral diabetic neuropathy

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Evidence for the important role of peroxynitrite in peripheral diabetic neuropathy (PDN), is emerging. Peroxynitrite causes protein nitration and multiple other consequences such as lipid peroxidation, DNA breakage and base modification, changes in cell signaling, PARP activation. This study evaluated the roles for iNOS, nNOS, and protein nitration in PDN. In experiment 1, protein nitration in sciatic nerve and DRG neurons, and PDN were compared in control (C) and STZ-diabetic (STZ-D) wild-type, iNOS-/-, and nNOS-/- mice. In experiment 2, C and STZ-D mice were treated with/without the protein nitration inhibitor epicatechin (10 mgkg-1d-1 i.p., for 4 wks after 2 wks without treatment). iNOS was found to play a key role in diabetes-induced protein nitration in peripheral nerve, whereas nNOS contributed to nitration in DRG neurons. iNOS gene deficiency completely protected from diabetes-induced MNCV and SNCV deficits and thermal hypoalgesia that were clearly manifest in STZ-D wild-type mice. Tactile allodynia and intraepidermal nerve fiber loss were alleviated. Non-diabetic nNOS-/- mice displayed reduced tactile response thresholds and ~29% reduction in intraepidermal nerve fiber density. STZ-D nNOS-/- mice developed similar SNCV deficit, tactile allodynia and small sensory nerve fiber degeneration as STZ-D wild-type mice, and were partially protected from MNCV deficit and thermal hypoalgesia. Treatment with epicatechin, 10 mgkg-1d-1, reduced protein nitration in both peripheral nerve and DRG neurons, preserved normal MNCV, and alleviated SNCV deficit, thermal hypoalgesia, and intraepidermal nerve fiber loss, but not tactile allodynia. In conclusion, iNOS, nNOS, and protein nitration in both peripheral nerve and DRG neurons contribute to PDN.

Hydroxytyrosol-rich olive mill waste water extract protects brain cells *in vitro* and *ex vivo*

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Elevated oxidative and nitrosative stress both impair integrity and functioning of brain tissue, especially in aging. As long-term intake of plant foods rich in antioxidant phenolics, such as extra virgin olive oil, positively modulate surrogate markers of many human pathological alterations, the interest in cheap and abundant sources of such phenolics is rapidly growing. Olive mill waste water is particularly rich in hydroxytyrosol, an ortho-diphenol with powerful antioxidant, anti-inflammatory, and anti-thrombotic activity. Considering the deleterious effect of oxidative stress on brain cell survival, we investigated the efficacy of a hydroxytyrosol-rich extract to attenuate Fe^{2+} and nitric oxide (NO)-induced cytotoxicity in murine dissociated brain cells. The addition of either Fe^{2+} or SNP (an NO donor) caused both a severe loss of cellular ATP and a markedly de-polarized mitochondrial membrane potential. Pre-incubation with hydroxytyrosol significantly attenuated the cytotoxic effect of both stressors, though with different efficiency. Mice feeding studies were performed to assess the brain bioactivity of hydroxytyrosol ex vivo. Sub-chronic, but not acute, administration of 100 mg hydroxytyrosol per kg body weight for 12 days enhanced resistance of dissociated brain cells to oxidative stress, as shown by reduced basal and stress-induced lipid peroxidation. Also, basal mitochondrial membrane potential was moderately hyperpolarized ($P < 0.05$), an effect suggestive of cytoprotection. In synthesis, our ex vivo data provide the first evidence of neuroprotective effects of oral hydroxytyrosol intake.

Reactive species augment blood-mediated inflammatory response

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Emerging concept suggests that blood is a central determinant for the involvement of reactive species (ROS) in cell signaling processes involved in coronary heart disease (CHD). We conducted a series of studies to identify key inflammatory pathways targeted by ROS and their implications in the pathogenesis of CHD. We at first demonstrated using an ex vivo model of blood recirculation that there is a greater imbalance between antioxidant capacity and the production of ROS in CHD patients as compared with healthy subjects. We also demonstrated an increasing pro-oxidant activity at different levels of disease progression from stable to unstable angina and impaired left ventricular function. Immunohistochemistry of myocardial biopsies demonstrated a significant differential distribution of apoptotic cell-death and an extensive infiltration of blood-derived monocytes into the vessel wall in patients with stable angina, unstable angina and impaired left ventricular function with greater levels in the latter two stages, a process that was significantly related to peroxynitrite-mediated NF-κB activation. Further in vitro investigations suggested that peroxynitrite regulated the production of inflammatory cytokines by monocytes via both canonical and non-canonical pathways of NF-κB activation and that NOS-derived endogenous nitric oxide acts as the major regulator of inflammatory pathways via NF-κB activation in isolated leukocytes from CHD subjects with or without type II diabetes mellitus.

Activation of equine neutrophils by phorbol myristate acetate or N-formyl-methionyl-leucyl-phenylalaline induces a different response in reactive oxygen species production and release of active MPO

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Neutrophil contribution to the acute inflammatory processes includes an excessive generation of reactive oxygen species (ROS) and secretion of granule enzymes. We compared the effects of either phorbol myristate acetate (PMA) or N-formyl-methyonyl-leucyl-phenylalanine (FMLP) with a pre-treatment by cytochalasin B (CB) on the production of ROS and the release of total and active myeloperoxidase (MPO) by isolated equine polymorphonuclear neutrophils (PMN). The ROS production was assessed by lucigenin dependent chemiluminescence (CL) and ethylene release from a-keto-g-methylthiobutyric acid (KMB) oxidation. In the supernatant of activated PMN, total equine MPO was measured by ELISA and active MPO by the original SIEFED technique*. The stimulation of PMN with CB-FMLP modestly increased the release of MPO, but more than 70 % of released MPO were active. PMA stimulation markedly increased the production of ROS and release of MPO, but more than 95 % of released MPO were inactive. When the PMN were pre-incubated with superoxide dismutase (SOD) before PMA stimulation, the lucigenin enhanced CL, linked to the •O2- production, was strongly and better decreased than the KMB oxidation, linked mainly to the hydroxyl-like radical production via H2O2, but, the released MPO remained active. These results confirm the key role of superoxide anion generation in the ROS cascade in PMN and reveal a critical protective role of SOD on MPO activity.

*Franck et al. (2006) J. Vet. Diagn. Invest. 18, 326-334.

Daily green tea extract consumption does not affect vascular reactivity or risk factors for coronary heart disease in healthy men

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Coronary heart disease (CHD) is one of the major causes of premature death in the western world. High concentrations of blood lipids and high blood pressure, caused by endothelial dysfunction, are established risk factors for CHD. On the other hand, high blood concentrations of antioxidants, including polyphenols and vitamin E, may be associated with a reduced risk for CHD. We conducted a double blind placebo controlled parallel study to evaluate the chronic effects of green tea polyphenols (GTP) on vascular reactivity, blood pressure, and blood lipids in healthy men. Volunteers (treatment, n=17, BMI 25.9±3.2 kg/m^2, age 41±9 y; placebo, n=16, BMI 24.8±3.0 kg/m^2, age 40±10 y) consumed for 3 weeks 6 gelatine capsules per day (2 capsules with each principal meal) containing aqueous extracts of the leaves of Camellia sinensis (equivalent to 119 mg GTP/d) or placebo. At the beginning and end of the intervention period fasting blood samples were collected and vascular tone was measured by Laser Doppler Iontophoresis. Analyses of endothelium-dependent and –independent vascular reactivity did not reveal differences between treatments. Neither resting blood pressure nor plasma concentrations of asymmetric dimethylarginine, total cholesterol, HDL cholesterol, triaclyglycerols, and tocopherols were affected by consumption of GTP. These data suggest that the consumption of green tea polyphenols for the duration of 3 weeks does not affect the elasticity of the vascular endothelium or other CHD risk factors in healthy men.

Engraulis encrasicolus vitamin E resource

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The objective of the present study was to investigate by high-performance liquid chromatography the effect of chilling storage, salting, marinating and frying on vitamin E levels in Engraulis encrasicolus muscle tissue using Kage-Vaugien et al., [1] method adapted in fish [2]. Salting and marinating was performed only on fresh fillet, while frying on fresh fillet and fillet after storage for 24h and 48h at 4°C. Our results show relative higher amount of vitamin E in muscle tissue of Engraulis encrasicolus (20.1 ± 2.2 µg/g) respect other "blue fish" (Sardina pilchardus, 5.70 ± 0.9 µg/g; Trachurus trachurus, 5.41 ± 0.4 µg/g; Scomber scombrus, 14.34 ± 2.0 µg/g) [3]. The vitamin E antioxidant resource decreases significantly during storage. A comparison levels between salted (24.0 ± 1.3µg/g), marinated (19.4 ± 1.0 µg/g) and fresh fish (20.1 ± 2.4 µg/g) demonstrates no significant differences, while a comparison of vitamin E levels between fresh fried (10.8 ± 1.7 µg/g), fried after 2h at 4°C (7.0 ± 1.6 µg/g) and fresh fish indicates that this resource in fried, decreases significantly. The relative amount detected could be associated with potential lipid peroxidation in post-mortem fish, and considered as an indirect index of oxidative stress effects. Salting and marinating procedures represent in Engraulis encrasicolus the possibility of good food preservation methods for maintaining higher the vitamin E resource.

[1] Kaye-Vaugien, C. et al. (1990) - Int. Vit. Nutr. Res., 60: 324-330.
[2] Ciarcia, G. et al. (2000) - Biofactors, 11: 19-21.
[3] Guerreiro G. et al. (2008) – J. Clinic. Biochem. Nutr., in press

Effects of dietary α-tocopherol from rapeseed oil on redox status and the expression of Alzheimer's disease-relevant genes in the cortex of rats

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Neurodegenerative disorders such as Alzheimer's disease (AD) are associated with systemic oxidative stress. While some intervention studies in humans indicate that dietary α-tocopherol, the major lipid-soluble antioxidant in plasma, may be effective in the prevention and treatment of AD, other clinical trials failed to confirm these beneficial effects. Rapeseed oil is an edible oil of high nutritional value and is recognized for its high concentrations of natural tocopherols (toc). Rapeseed oils with increasing concentrations of α-toc were produced and used to prepare diets with deficient, marginal, sufficient and high (<1, 8, 15, and 151 mg/kg) concentrations of natural-source (RRR) α-toc. Feeding of the experimental diets for 6 months dose-dependently increased α-toc concentrations in cortex and plasma of male Fisher rats (8 rats per group). 151 mg α-toc/kg diet significantly lowered tissue levels of γ-toc. Concentrations of F2- isoprostanes in the cortex were not significantly different between the 4 groups. The activities of the antioxidative enzymes superoxide dismutase and glutathione peroxidase as well as the concentrations of glutathione were measured in cortex homogenates and not affected by the α-toc content of the diet. Gene expression of amyloid beta (A4) precursor protein (APP), APP binding family member 1, Adam 10, Bace 1, acetylcholinesterase, Bcl-2, and Bax, measured in cortex and hippocampus, did not differ depending on the α-toc content of the diet. Overall, current data suggest that the intake of increasing levels of α-toc for 6 months did not result in significant changes in redox state or expression of genes relevant in the development of AD.

Astrocytes protect PC12 cells from dopamine-induced neuronal death

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Dopamine is an archetypical neurotransmitter that is integral in the motor circuit pathway, however its molecular structure (catechol moiety) makes it unstable and easily oxidizable. Its neural toxicity properties have been well documented. To limit dopamine toxicity, once synthesized dopamine is actively pumped into synaptic vesicles, and stored until released as a neurotransmitter. Synaptic vesicles not only provide a physical barrier to protect dopamine from enzyme degradation, but its acidic environment favor the protonated over the de-protonated form of dopamine, which is more prone to (auto)oxidation, and consequently superoxide generation. Recent studies have suggested the leakage of dopamine from the synaptic cleft. This seepage allows oxidation of dopamine as well as contact with surrounding glial cells (astrocytes). This present study delves into the response primary astrocytes have to dopamine, with respect to dopamine oxidation/toxicity, neuronal protection, and inflammation. Utilizing a co-culture system, we demonstrated that astrocytes protect PC12 cells from dopamine toxicity. Astrocytes prevent auto-oxidation of dopamine as shown by a decrease in dopaminochrome formation. An interesting consequence of dopamine exposure to astrocytes is the up-regulation of iNOS by a H_2O_2 dependent pathway. Whether dopamine induced up-regulation of iNOS in astrocytes play a significant role in the development of PD remains to be further investigated.

The influence of substrate availability on redox and energy status of brain mitochondria

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Glutathione (GSH) is the most abundant non-protein thiol in cells and plays a major role in regulating protein redox status. Mitochondria contain a separate GSH pool, the depletion of which has profound consequences for cell viability and/or dysfunction. However, the regulation of mitochondrial GSH is not well understood. In particular, factors that regulate mitochondrial GSH redox status, which would have profound effects on mitochondrial protein redox status (e.g. disulfide bound formation, glutathionylation), have not been well characterized. This present study focuses on the role of mitochondrial substrate generated reducing equivalents in modulating the GSH homeostasis and protein glutathionylation in mitochondria. The availability of reducing equivalents had profound effects in maintaining the mitochondrial GSH and proteins in a reduced state in response to oxidative stress. H_2O_2 dependent glutathionylation was inhibited when complex I substrates were available. Additionally, mitochondrial redox status was found to be dependent on substrate availability and independent of mitochondrial respiration. This study contributes to the further understanding of how mitochondrial redox status is regulated and that mitochondrial substrates not only contribute to ATP generation but also to regulating/protecting overall mitochondrial function through (de)glutathionylation.

Modified LDL activates JNK-2 phosphorylation and co-localization with mitochondria

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Modified LDL initiates the pathogenesis of atherosclerosis. JNK-2 protein kinase (SAPK) knockout mice and CD36 knockout mice harbor a decrease in macrophage foam cell formation and the CD36 knockout mice have decreased p-JNK-2 upon oxLDL treatment. We hypothesized that protein unfolded LDL induces an oxLDL-receptor-dependent phosphorylation of JNK and the induction of apoptosis. We assessed whether modified LDL activates JNK-2 phosphorylation, induces its co-localization with translocation to the mitochondria and induction of mitochondrial-driven apoptosis. Modified LDL particles (10mg/ml) were incubated with bovine aortic endothelial cells (BAEC) in the presence or absence of JNK inhibitor. Western blot analyses showed a 1.50 ± 0.24-fold, 1.39±0.18-fold increase and a 1.32 ± 0.11-fold increase in p-JNK-2 in response to copper-, $ONOO^-$- and PLA_2-treated LDL (n = 3, $P < 0.05$) respectively. Modified LDL induced p-JNK-2 localization to the mitochondria which was inhibited by JNK inhibitor. We also found that upon receptor blocking to both CD36 and SR-A, JNK-2 phosphorylation was ablated, mitochondrial co-localization was ablated and the subsequent phosphorylation of Bcl-x_L and caspase-3 activities ablated but incubation with either receptor blocking antibody alone was incapable of this inhibition suggesting that both receptors are involved. Our findings demonstrate that protein unfolded LDL induces an oxLDL-receptor-dependent JNK-2 phosphorylation, its co-localization to the mitochondria, and subsequent activation of apoptosis and suggest a mechanism to increasing necrotic core size in atherosclerosis.

Peroxidase systems in *Helicobacter pylori*

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Helicobacter pylori is a wide-spread and important human pathogen, since persistent infection contributes to the development of stomach cancer. While *H. pylori* infection is generally controlled with a cocktail of antibiotics, the emergence of antibiotic resistance necessitates the search for alternative therapeutic strategies. Like other pathogens *H. pylori* has to defend itself against the oxidative attack of the host's innate immune response. However, *H. pylori* lacks glutathione and, consequently, the glutathione peroxidases that dominate the mammalian antioxidant defense. The peroxide metabolism of the pathogen has for long been recognized to depend on catalase and superoxide dismutase. Deletion of the gene encoding catalase did not affect the viability of the organism. Accordingly, survival and virulence are attributed to the peroxiredoxin-type peroxidases alkyl hydroperoxide reductase (AhpC) and "thiol peroxidase" (TPx), which reduce a variety of hydroperoxides. However, the most common AhpC reductant in bacteria, the disulfide reductase AhpF, is also absent in *H. pylori*. Instead, AhpC and TPx can be reduced by one of the bacterial thioredoxins, Trx1. *H. pylori* contains another peroxiredoxin, Bcp and a second thioredoxin, Trx2, whose functions still remain unclear. In short, the antioxidant defense system of *H. pylori* differs substantially from that of the mammalian host. Its constituents may therefore be regarded as potential drug targets. Selective inhibition of the pathogen's defense systems will enhance the efficacy of the innate immune response without adversely affecting the host.

Myeloperoxidase-dependent nitration and oxidation of *p*-cresol: A potential novel class of inflammation-derived toxins

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p-Cresol (4-methylphenol) is a recognized toxin in end-stage renal disease. However, its potential role in lung disease including patients with chronic obstructive lung disease is unknown. COPD patients can be exposed to *p*-cresol via exposure to ambient pollutants, primarily through car exhaust and cigarette smoke, and from endogenous sources via metabolism of tyrosine by gut microflora. While inflammation and oxidative stress are common hallmarks of COPD, it is not known whether compounds in smoke, except for benzo[*a*]pyrene, can be chemically modified by oxidative processes to produce more potent toxins. Herein we demonstrate, using chemical and enzymatic systems, that *p*-cresol is converted into nitrated, chlorinated and oxidized metabolites by myeloperoxidase-derived oxidants. Chemical experiments demonstrate that *p*-cresol can be converted into nitrated, chlorinated and oxidized metabolites by hypochlorous acid ($HOCl/OCl^-$) and nitryl chloride (NO_2Cl). Enzymatic experiments with MPO and H_2O_2 resulted in the bioconversion of *p*-cresol to 4a,9b-dihydro-8,9b-dimethyl-3(4H)-dibenzofuranone and 2,2'-dihydroxy-5,5'-dimethyldiphenyl but not chlorinated products. These novel oxidative products of *p*-cresol (and presumably other aromatic substrates) by MPO may represent novel inflammation-derived toxins that can collectively be termed 'inflammatoxins'. These products may link inflammation and oxidative processes to environmental toxins and may have pathological consequences.

Lipoic acid regulates glutathione and heme oxygenase gene expression as a mechanism of protection against arsenic exposure in HEPG2 cells

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Oxidative stress is one of the mechanisms of arsenic toxicity. Cellular response is done by the synthesis of protective compounds and stress response proteins like glutathione (GSH) and heme oxygenase-1 (HMOX1). Modulation of the antioxidant response is a key factor for the survival of the cell. Alpha-lipoic acid (ALA) is a well recognized antioxidant that has shown a beneficial role in several metabolic diseases and chemical intoxications. In this work we have analyzed the mechanism of protection of ALA treatment on HepG2 cells subsequently exposed to several arsenic concentrations. METHODS. HepG2 cells were treated with 5 mM ALA and 8 h later exposed to sodium arsenite (As) at 50, 100 or 150 mM for 24 h. Cells were analyzed for metabolic MTT activity, GSH, and key antioxidant gene expression by qRT-PCR. RESULTS. 50 mM As evoked a strong cellular response increasing GSH content (134%) and HMOX1, GCLM and MTA1 gene expression (35-, 10- and 9- fold of control cells, respectively), but at higher doses As was lethal to cells. A dose-response reduction in MTT activity and GSH were observed by As exposure, however ALA prevented these decreases by maintaning a constant level in both parameters at all As doses. Interestingly, with the protection afforded by ALA there was a reduction of the strong HMOX1 response elicited by 50 mM As alone (from 35- to 5-times of control cells). CONCLUSIONS. HepG2 cells exposed to low As concentrations can efficiently manage the toxicity by increasing an antioxidant response. ALA pretreatment can down-modulate that response effectively protecting the cells against arsenic.

Selective estrogen receptor modulators potentiate brain mitochondrial function

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We have previously shown that 17β-estradiol (E_2) regulates the mitoproteome to potentiate the function of mitochondria isolated from whole brain (Nilsen, Irwin et al. J. Neurosci 2007). The purpose of this study was to determine the role of the ER subtypes in regulation of mitochondrial function using the ER subtype selective agonists PPT (ERα) and DPN (ERβ). Adult ovariectomized rats were treated with E_2 (30 μg/kg), PPT (30 μg/kg), DPN (100 μg/kg) or vehicle control 24 h prior to isolation of whole brain mitochondria by discontinuous Percoll density centrifugation. A Clarke-type electrode was used to record mitochondrial oxygen consumption. After 24 h all estrogenic treatments significantly increased mitochondrial respiration relative to vehicle control as measured by the respiratory control ratio (RCR). Likewise, cytochrome c oxidase activity increased following all estrogenic treatments. PDH activity and production of H_2O_2 by isolated mitochondria correlated with changes in respiration. RT-PCR and Western blots of cortex and hippocampus homogenates were performed for COX II (mtDNA) and COX IV (nucDNA) expression. Lipid peroxides were reduced by E_2, PPT, and DPN. These findings suggest that activation of both ERα and ERβ enhance mitochondrial function in brain. Future synthetic estrogens and phytoestrogens used in hormone therapies may be tailored to improve mitochondrial endpoints. We are currently following up these investigations in cell cultures and transgenic mouse models relevant to neurodegenerative diseases.

H_2O_2 induced contraction in HFF STZ induced diabetic rat thoracic aorta: Role of GSK-3

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The primary goal of this project work was to decipher precise cellular events linking to type-II diabetes mellitus and vascular dysfunction with main emphasis on GSK-3. Here, H_2O_2 was used as a tool to find out the role of GSK-3 in diabetes induced vascular dysfunction. Diabetes was induced by high fat diet and low dose of STZ in rats. Seven week old diabetic rats were performed for vascular reactivity study. Contractile responses of H_2O_2 (10-6 M to 10-3 M) were taken in spiral rat thoracic aorta in normal and diabetic rats. Lithium chloride and sodium valproate were used as a GSK-3 inhibitors. Vascular dysfunction in diabetes is developed due to the development of oxidative stress. The production of oxidative stress is confirmed by the measurement of antioxidant levels in diabetic rats. From the results of blood glucose, insulin, SOD, catalase and MDA it is confirmed that GSK-3 inhibitors have anti-hyperglycemic and antioxidant activity. Inhibition of H_2O_2 (10^{-6} M to 10^{-3} M) induced contractile response in rat thoracic aorta with lithium chloride and sodium valporate indicates the role of GSK-3 in H_2O_2 induced contraction. It was clearly demonstrated from our study that the inhibition of GSK-3 activity, inhibited augmented vascular responses to H_2O_2 in diabetic rat thoracic aorta.

Zinc and the modulation of MAPK and Akt in the nervous system

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We previously demonstrated that a low Zn availability triggers neuronal apoptosis in association with an altered modulation of the mitogen activated kinases (MAPKs) ERK1/2, p38, and JNK. We have now investigated the effects of feeding diets containing adequate (25 µg Zn/g, C) or marginal zinc (10 µg/g, MZD) concentrations throughout gestation on the modulation of the protein kinases MAPKs and Akt in E19 rat fetal brain. Food intake, pregnancy and fetal outcome were similar between groups. A differential activation of MAPKs and Akt, as evaluated by phosphorylation levels, was observed. Low levels of ERK1/2 activation and high levels of JNK and p38 activation were measured in fetal brain from the MZD group compared to the C group. Akt phosphorylation levels were higher in MZD fetal brain than in controls. Given the role of ERK and Akt in the inhibition of mitochondria-mediated apoptosis, the activation of both kinases was next evaluated in primary cortical neurons. A similar pattern of decreased ERK phosphorylation and high Akt phosphorylation was observed in cells incubated in zinc deficient media compared to controls. These results show that a marginal zinc nutrition imposed on rats during gestation affect MAPKs and Akt modulation in E19 fetal brain with a similar pattern to that observed in zinc deficient neuronal cells. Given the general pro-survival roles of ERK and Akt, their relative relevance in the neuronal apoptosis triggered by a low Zn availability deserves further investigation.

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Lycopene attenuates diabetes associated cognitive decline in rats

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Experimental evidences suggest that encephalopathy, characterized by acquired cognitive and behavioural deficits, can occur as a late complication of diabetes. The present study was designed to investigate the effect of lycopene, a potent antioxidant and anti-inflammatory molecule, on cognitive functions, oxidative stress and inflammation in diabetic rats. Cognitive functions were investigated using a spatial version of the Morris water maze test. Acetylcholinesterase activity, a marker of cholinergic dysfunction, was increased by 1.8 fold in the cerebral cortex of diabetic rats. There was about 2 fold and 2.2 fold rise in thiobarbituric acid reactive substance levels in cerebral cortex and hippocampus of diabetic rats, respectively. Non protein thiol levels and enzymatic activities of superoxide dismutase and catalase were decreased in both cerebral cortex and hippocampal regions of diabetic rat brain. Total nitric oxide levels in cerebral cortex and hippocampus was increased by 2.4-fold and 2-fold respectively. Serum tumor necrosis factor-alpha, an inflammatory marker, was found to increase by 8 fold in diabetic rats. Chronic treatment with lycopene (1, 2 and 4 mg/kg; p.o.) significantly and dose-dependently attenuated cognitive deficit, increased acetylcholinesterase activity, oxidative-nitrosative stress and inflammation in diabetic rats. The results emphasize the involvement of oxidative-nitrosative stress and inflammation in the development of cognitive impairment in diabetic animals and point towards the therapeutic potential of lycopene in diabetes-induced learning and memory impairment.

Neuronal nitric oxide synthase enhances paraquat cytotoxicity during aging by functioning as a paraquat diaphorase

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Parkinson¡¦s disease is a progressive neurodegenerative disorder that is characterized by the loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) region of the midbrain. Large scale epidemiologic studies unraveled a strong correlation between paraquat exposure and increased risk to Parkinson¡¦s disease. Cytotoxicity mediated by paraquat can be attributed to its ability in producing superoxide ($O_2^{\cdot-}$) by redox cycling with a cellular diaphorase. One of the possible diaphorases is nitric oxide synthase (NOS), which is a flavin- and heme-containing enzyme that catalyzes the metabolism of L-arginine to L-citrulline and $^{\cdot}NO$ in the presence of O_2 and NADPH. Electrons involved in the process can be diverted to paraquat reduction to paraquat radical ($PQ^{\cdot+}$) and subsequent $O_2^{\cdot-}$ formation upon redox cycling. In this study, differentiated PC12 dopaminergic cells and brain homogenates from rats of different ages were used to study: *1)* effect of paraquat on dopaminergic cells viability and cytotoxicity, *2)* the inherent role of neuronal NOS (nNOS) in mediating paraquat-toxicity by acting as a paraquat diaphorase and *3)* the correlation between age-dependent increase in nNOS level and the susceptibility of dopaminergic neuron to paraquat induced cytotoxicity. Data obtained in this study demonstrated that nNOS functions as a paraquat diaphorase that facilities the formation of paraquat radical in a NADPH-dependent manner, thus increasing the susceptibility of dopaminergic cells to environmental toxin-induced apoptosis.

Role of neuronal nitric oxide synthase in JNK-mediated neurodegeneration: Implication for Parkinson's disease

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Nitric oxide synthase (NOS) is a flavin- and heme-containing enzyme that catalyzes the metabolism of L-arginine to L-citrulline and nitric oxide ($^{\cdot}NO$) in the presence of O_2 and NADPH. Neuronal NOS (nNOS) is a Ca^{2+}-calmodulin-dependent isoform of NOS that is constitutively expressed in neuronal cells. The cellular level of nNOS is regulated by its turnover through degradation by the proteasome. Dysfunctional proteasome is often implicated in Parkinson¡¦s disease in which the formation of Lewy bodies and a progressive degeneration of dopaminergic neurons are observed. Nitrated proteins are accumulated in Lewy bodies indicating an enhanced $^{\cdot}NO$ production and reactive nitrogen species formation. However, to date, there has been no extensive study undertaken to investigate the role of proteasome function and nNOS level with respect to viability of dopaminergic neuron. In this study, proteasome dysfunction is induced by treating PC12 dopaminergic cell model with the proteasome inhibitor MG132. It was shown that *1)* impairment of proteasome activity induces upregulation of nNOS protein, *2)* enhanced expression of nNOS is linked to increased $^{\cdot}NO$ and $ONOO^-$ levels. *3)* $ONOO^-$ activates c-Jun N-terminal kinase (JNK) that induces inhibition of Bcl-x_L (antiapoptotic Bcl-2 family member) by phosphorylation and eventually triggers the activation of a downstream apoptosis cascade that includes the commitment (caspase-9) and execution (capase-3) phases. *4)* Addition of either general or specific nNOS inhibitors inhibits apoptosis, thus further strengthening the role of nNOS in .NO mediated-neurodegeneration induced by proteasome impairment.

Mitochondrial protein nitration: implications for an impaired energy metabolism during aging

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Neuronal nitric oxide synthase levels in brain homogenates increase with aging and is accompanied by an increase in mitochondrial protein nitration. This suggests the involvement of $ONOO^-$ in the latter process, supported by the increased .NO generation and mitochondrial superoxide anion production. Nitration of two important brain mitochondrial proteins was found as a function of age: F_1-ATPase (Complex V) and succinyl-CoA transferase (SCOT). The former protein generates ATP through the protonmotive forces and the latter is critical for ketone body metabolism, an alternative energy source during impaired or inefficient glucose metabolism in brain. Nitration of F_1-ATPase at Tyr^{269} (identified by LC/MS/MS) caused a decreased in activity; likewise, nitration of SCOT was associated with decreased activity. Furthermore, the decrease in their activities as a function of aging was more pronounced than that of cytochrome *c* oxidase (complex IV), an indicator of mitochondrial respiration. These data suggest that mitochondrial dysfunction with aging can be attributed to increase protein nitration (F_1-ATPase and SCOT) that decreases the generation of reducing equivalents (from ketone bodies) and compromises oxidative phosphorylation. These protein post-translational modifications are associated with an enhanced cytochrome *c* release into the cytosol, thus suggesting a linked between impaired mitochondrial function and mitochondrion-driven apoptosis.

Matrix metalloproteinase-2 activity in human aortic endothelial cells is regulated by the calpain/calpastatin system: Implications for estrogen based therapies

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The calpain/calpastatin system regulates the gelatinases matrix metalloproteinase-2 (MMP-2) and MMP-9 in leukemic cell invasion; however, the regulation of these enzymes in vascular cells are unknown. This study was aimed at characterizing the role of the calcium-dependent cysteine protease calpain-1, calpain-2, and their specific inhibitor calpastatin in the expression of MMP-2 in human aortic endothelial cells (HAEC). Treatment with 17£]-estradiol (E2) was performed in HAEC and MMP-2 activity was measured by gelatin zymography. Calpain-1, calpain-2, and calpastatin protein levels were measured by western blot in cells lysates. MMP-2 activity decreased in HAEC treated with E2 ($p<0.0005$). There was no difference in calpain-1 protein levels of HAEC treated with E2 as compared with control cells. However, calpain-2 protein levels were decreased ($p<0.0005$) in response to E2 treatment and this decrease was accompanied by an increase ($p<0.005$) in calpastatin levels. These data indicate for the first time that calpain-2 and calpastatin play a role in modulating the expression of MMP-2 gelatinase activity in human vascular cells. The decrease in MMP-2 expression from human vascular cells in response to E2 is an important observation for E2 has been demonstrated to increase MMP-9 expression from macrophages, suggesting that E2 action in relation to metalloproteinase activity varies according to cell model and system. Understanding the interaction of E2 with the mechanism of MMP regulation may help unravel the mechanistic basis underlying the effects of E2 on plaque rupture in healthy and diseased vascular tissues and possibly the development of therapeutic approaches for preventing plaque rupture.

PTEN regulates mitochondrial energy production

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PTEN (Phosphatase and Tensin homolog) is a human tumor suppressor gene which is involved in the regulation of the cell cycle and cell growth. PTEN mutations and deletions are frequently found in various cancers, however, tumorigenesis is a complicated process and the mechanism of how PTEN mutations or deletions contribute to tumorigenesis is not completely understood. We hypothesize that PTEN-knockout liver tissue has increased mitochondrial function that produces extra energy for increased cell proliferation and fatty acid synthesis, thus leading to cell cycle dysregulation, tumorigenesis and fatty liver. Mitochondria were isolated from the liver of wild type and PTEN-knockout mice by differential centrifugation and then purified by percoll gradient. Mitochondrial Respiratory Control Rate (RCR) and mitochondrial ATP synthase activity was measured. Cell growth was also observed in the cultured wild type and PTEN knockout liver cell lines. Our preliminary data showed that PTEN knockout liver mitochondria had a higher RCR and ATP synthase activity than that of wild type liver mitochondria. We also observed that PTEN knockout liver cells grow faster than wild type liver cells, which suggests an aberrant increase in the cell proliferation. We also found that the expression of PDH-E1¦Á was increased. PTEN-knockout mice developed fatty livers, indicating the increased fatty acid synthesis in liver.

The essential role of non-mitochondrial form of Gpx4 in mouse development

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Glutathione peroxidase 4 (Gpx4) is an antioxidant enzyme that repairs oxidative damage to biomembranes. Our previous study in Gpx4 knockout mice indicated that Gpx4 is essential for survival of mice and deficiency in Gpx4 renders cells and mice prone to oxidative stress. In addition, our previous results from Gpx4 transgenic mice showed that increase in Gpx4 protects against oxidative stress and ameliorates Ab induced toxicity in neurons. Gpx4 also plays important roles in regulating the mitochondrial pathway of apoptosis and protecting mitochondrial function from ROS induced damage. Somatic cells have two forms of Gpx4: a mitochondrial form and a non-mitochondrial form, both of which are derived from the same gene. The two forms of Gpx4 have been implicated to play different roles in antioxidant defense, apoptosis and development. To illustrate the biological functions of the two forms of Gpx4, we have generated transgenic mice that overexpress either mitochondrial Gpx4 or non-mitochondrial Gpx4. Our results from these mice indicate that the non-mitochondrial Gpx4 is the essential form of Gpx4 for mouse development and survival, as evidenced by the fact that the non-mitochondrial form of Gpx4 is able to rescue the lethal phenotype of Gpx4 null. Thus, the transgenic mice overexpressing mitochondrial Gpx4 or non-mitochondrial Gpx4 are valuable animal models to determine the different roles of Gpx4 in mammalian systems.

Induction of a mild diet-induced oxidative stress and effects of quercetin (Q) on the α-tocopherol (TOC) and oxidative status in piglets

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The aim of this study was to investigate possible Vit E-sparing effects of Q in pigs fed diets with a low TOC content with or without the addition of fish oil to induce a mild dietary oxidative stress. 26 male, castrated pigs with an initial mean body weight (BW) of 9.9 kg were used. During the depletion period (5 weeks) all pigs were fed a low TOC diet (C). Thereafter, animals were assigned to one of 4 feeding groups receiving C (n = 7), C + 10 mg Q/kg BW/day (CQ; n = 7), C supplemented with 5% fish oil (F; n = 6) or F + 10 mg Q/kg BW/day (FQ; n = 6) for 4 weeks. The plasma TOC concentrations, thiobarbituric acid reactive substances (TBARS) and 8-isoprostaglandin-F2α (8-iso-PGF2α) were measured. At the end of the intervention period, plasma TOC was significantly reduced in groups F and FQ compared to controls (C, CQ) concomitant with a significant increase in plasma TBARS and 8-iso-PGF2α concentrations. Without feeding fish oil, supplementation of Q elevated plasma TOC concentration ($p < 0.05$) with no effect on TBARS and plasma 8-iso-PGF2α (C vs. CQ). In animals receiving fish oil, Q ameliorated the increase of TBARS and of the plasma concentration of 8-iso-PGF2α ($p < 0.05$). Hence, at low dietary Vit E intake, a Vit E-sparing effect of Q could be demonstrated. Addition of fish oil to the low Vit E diet induced a measurable oxidative stress indicated by a significant increase in TBARS and the 8-iso-PGF2α plasma concentration as well as a further reduction of plasma TOC. Under these conditions, however, evidence for a Vit E-sparing effect of Q was not obtained but the decrease of markers of oxidative stress points to a Vit E-independent antioxidative mechanisms of Q.

Zinc and the cytoskeleton of neuronal cells

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Zinc deficiency impairs tubulin polymerization in neuronal cells. This can have major adverse consequences on cell function, including an altered nuclear transport of select transcription factors (NF-κB and NFAT). This work investigated the potential mechanisms involved in the disruption of the cytoskeleton when neuronal zinc decreases. Zinc deficiency causes a decreased rate of in vitro tubulin polymerization in human neuroblastoma IMR-32 cells and in brain supernatants from GD19 fetuses fom dams fed marginal zinc diets. The incubation of IMR-32 cells for 24 h in low zinc media led to an altered distribution of cellular tubulin, and a decreased amount of polymerized tubulin. When cells were incubated in zinc deficient media and in the presence of N-acetylcisteine (NAC) or α-lipoic acid (LA), the rates of tubulin polymerization reached control values. The presence of tubulin aggregates in non-reducing condition was investigated by Western blot. Tubulin aggregates of MW higher than 100 kDa were observed in the supernatants isolated from zinc deficient cells, that disappeared in the cells incubated with NAC or LA. Furthermore, the addition of the thiol reductant TECP to supernatants from zinc deficient cells or GD19 fetal brain restored the rates of tubulin polymerization. The obtained results support the possible involvement of oxidative modifications of tubulin as the underlying cause of the altered neuronal microtubule assembly occurring when neuronal zinc decreases.

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Space radiation-induced memory dysfunction and oxidative damage. Prophylactic action of α-lipoic acid

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To investigate the neurobehavioral consequences of whole-body charged particle irradiation such as might occur for astronauts during space flight, mice were exposed to high-LET 56Fe beams (500MeV/nucleon, 1.5Gy). Radiation exposure substantially impaired the reference memory at 30 day post-irradiation; however, no significant effect was observed on motor activities of mice. Pretreatment of mice with alpha-lipoic acid prior to irradiation significantly attenuated such memory dysfunction. Radiation-induced apoptotic damage in cerebellum was examined using a neuronal-specific terminal deoxynucleotidyl transferase-mediated nick end-labeling method (NeuroTACS). Radiation-induced apoptotic and necrotic cell death of granule cells and Purkinje cells were inhibited significantly by α-lipoic acid pretreatment. α-Lipoic acid pretreatment exerted a very high magnitude of protection against radiation-induced augmentation of DNA damage (comet tail movement and serum 8-OHdG), lipid proxidation products (MDA+HAE) and protein carbonyls in mice cerebellum. Further, radiation-induced decline of non-protein sulfhydryl (NP-SH) contents of cerebellum and total antioxidant capacity (TAC) of plasma was also inhibited by α-lipoic acid pre-treatment. Results clearly indicate that α-lipoic acid is a potent neuroprotective antioxidant. Moreover, present finding also support the idea suggesting the cerebellar involvement in cognition.

Nutraceutical strategy in aging: targeting heat shock protein and inflammatory profile via IL-6 polymorphism understanding

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The study was aimed to assess the inflammatory profile and polymorphism of healthy elderly subjects and the influence of nutraceutical supplementation. Forty aged subjects were divided in a cross-over manner in two matched groups given: A) a certified fermented papaya preparation (FPP, ORI, Gifu, Japan) 9g/day by mouth; B) same amount of placebo. Ten healthy young subjects served as control. IL-6 promoter-174 G/C polymorphism genotype was determined together with blood level of: Redox Status, pro-inflammatory cytokines, hs-CRP and serum Hsp70 concentration. The IL6 -174 genotype frequencies of CC (20%), CG (45%), and GG (35%) were in accordance with what described in Caucasian controls. TNF-a and IL-6 were higher in elderly subjects ($p<0.05$ vs young). All cytokine parameters were significantly more elevated in -174 GC genotype aged subjects ($p<0.05$). FPP significantly improved cytokine parameters ($p<0.01$) with values comparable to young subjects. The concentration of Hsp70 inversely correlated with markers of inflammation in -174 G/C-negative subjects (r: 0.62, $p<0.05$). Nutraceutical intervention normalized the inflammatory parameters ($p<0.05$) with a rise of Hsp70 ($p<0.05$). This suggests that healthy elderly may have a pro-inflammatory profile playing as a down-regulating factor for inducible Hsp70, expecially if -174 G/C-negative. The present nutraceutical intervention caused a significant parallel increase of Hsp70 and this phenomenon occurred in those subjects with a genomic risk profile.

Targeting heat shock protein and inflammatory profile through nutraceutical intervention: The role of IL-6 polymorphism understanding

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Ageing is accompanied by 2-4-fold increases in plasma/serum levels of inflammatory mediators such as cytokines and acute phase proteins. The study was aimed to assess the inflammatory profile and polymorphism of healthy elderly subjects and the influence of nutraceutical supplementation. Forty aged normofolemic male subjects were divided in a cross-over manner in two matched groups given: A) a fermented papaya preparation 9g/day by mouth; B) same amount of placebo. Major invalidating diseases were regarded as exclusion criteria as well as signs of dementia. A dietary questionnaire was used and habitual intake of macronutrient and micronutrient was also submitted using a seven-day diet history model. Ten healthy young subjects served as control. IL-6 promoter-174 G/C polymorphism genotype was determined together with blood level of: Redox Status, pro-inflammatory cytokines, hs-CRP and serum Hsp70 concentration. The IL6 -174 genotype frequencies of CC (20%), CG (45%), and GG (35%) were in accordance with Hardy-Weinberg equilibrium as described in Caucasian controls. TNF-α and IL-6 were higher in elderly subjects ($p<0.05$ vs young). The concentration of Hsp70 inversely correlated with markers of inflammation in -174 G/C-negative subjects (r: 0.62, $p<0.05$). Nutraceutical intervention normalized the inflammatory parameters ($p<0.05$) with a rise of Hsp70 ($p<0.05$). This suggests that healthy elderly may have a pro-inflammatory profile playing as a down-regulating factor for inducible Hsp70, expecially if -174 G/C-negative. A nutraceutical intervention seems able to beneficially modulate such phenomenon.

Antioxidants restore thiol metabolism alterations in rd1 retina

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Introduction: Retinitis pigmentosa (RP) is a group of inherited disorders characterized by progressive peripheral vision loss and night vision difficulties. Antioxidants are known to rescue photoreceptors in different models of RP, including rd1 mouse. Our aim was to understand possible mechanisms engaged by antioxidants and therefore we have analyzed levels of oxidative stress markers in rd1 retina when treated with antioxidants. Methods: rd1 mice were treated daily from postnatal (PN)3 orally with zeaxanthin, lutein, a-lipoic acid (ALA), glutathione GSH and Lycium barbarum extract until PN10. At PN 11 eyes were dissected, one retina was cryosectioned and the other homogenized in prechilled potassium phosphate buffer. This homogenate was used to assay GSH peroxidase (GPx) and GSH reductase (GSSG-R) activity and malondialdehyde (MDA), GSH and other thiol derivatives content. Results: Treatment with antioxidants significantly reduced the number of TUNEL-positive cells. There were no differences between MDA values and GSSG-R activity in control and rd1 retina. GPx activity was significantly decreased in retina from rd1 mice vs controls. GSH and (cysteine) Cyss levels were decreased and increased respectively in the untreated rd1 retinas vs controls. Treatment with antioxidants restored these alterations. Conclusion: Antioxidants reduce cell death in rd1 retina. Interestingly antioxidants combination used in this study has the ability to increase GSH concentrations and also GPx activity in the retina of rd1 animals. The mechanism of GSH depletion in retinal cells and its relation with apoptosis in rd1 retina is a challenge for future studies.

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Thermal burn injury in pediatric patients depletes body stores of vitamin E

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We hypothesized that the severity of the oxidative stress of thermal burn injury up-regulates inflammatory responses such that increases in both superoxide and nitric oxide production occur. These inflammatory responses prolong radical-mediated damage beyond that caused by the initial injury and potentially cause damage to organs distant to the initial injury. Moreover, this continued production of radicals could deplete antioxidant defenses, especially low molecular weight antioxidants. Although our group has previously demonstrated that thermal injury depletes plasma vitamin E, plasma changes may reflect immediate alterations in vitamin E nutriture. Adipose tissue a-T concentrations change slowly over time (years) and are generally accepted to reflect long-term vitamin E status. To test the hypothesis that thermal injury depletes body stores of vitamin E, adipose tissue was collected surgically at various intervals from 8 pediatric patients over the first 30 days post burn injury. During the first week following injury, a-T concentrations were 199 ± 105 nmol/g (mean ± SD), during the second week they decreased to 133 ± 32 and during the third week they decreased further to 109 ± 16. The rates of decrease and the estimated adipose tissue vitamin E concentration prior to injury were estimated by linear regression for each of the subjects. A-T/g adipose tissue decreased at an average rate of 2.5 nmol/g, such that at the end of a month nearly 100 nmol were lost, consistent with the actual measurements. These data emphasize the initial severity of the oxidative stress that the patients experienced and further suggest that oxidative damage in the form of lipid peroxidation continues to deplete vitamin E during the month following injury.

Free radical reaction and oxygen consumption in an aqueous solution by an X-ray irradiation

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Introduction: High LET carbon ion beam cancer therapy at National Institute of Radiological Sciences (Chiba, Japan) began in 1994. Considering the expansion of heavy-ion cancer therapy, the quality and accuracy of the heavy-ion therapy must be improved and optimized. Detection of free radical reactions and geometry in a large sample irradiated by a heavy-ion beam is thus important to understand the effect of high LET irradiation in patients. Therefore, a new method to detect free radical generation, which can be translated to an imaging technique, is required. In this experiment, using an X-ray irradiation, the imaging-translational detection of H_2O_2 generation in an aqueous sample was tested. *Method:* LiPc crystal was put into a plastic microtube. The microtube was filled with mili-Q water or PBS, and sealed air-tightly without any bubble in the tube. The tube was irradiated by X-ray (Eeff = 80 keV) with dose of 256 Gy. After the irradiation, EPR spectra of LiPc in the tube were measured at L-band (1.2 GHz). The pO_2 value in the tube was estimated from the linewidth of EPR spectrum of LiPc. A similar sample without LiPc was irradiated by X-ray (256 Gy), and then H_2O_2 concentration in the tube was measured. A water solution of TEMPOL (2 mM) was irradiated by X-ray (356 Gy), and the reduction of the EPR signal of TEMPOL was measured. *Results and Discussion:* By irradiating 256 Gy of X-ray, pO_2 in the irradiated water was decreased by 30 mmHg, and generation of 60 mmol/L H_2O_2 in the irradiated water was detected. When a water solution of TEMPOL (2 mM) was irradiated by 256 Gy of X-ray, EPR signal of TEMPOL in the sample was decreased. The H_2O_2 generated in the water during the irradiation can make the reduction of TEMPOL without hydrogen-donor, such as GSH, NADH, or NADPH. This experimental design will be translated to imaging experiment and applied to the high LET radiation such as heavy-ion beams in the future.

The effects of α-lipoate on NFκB activation and gene expression in oral keratinocytes after periodontitis-associated-bacterial stimulation

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The H400 oral epithelial cell line was cultured in the presence and absence of a-lipoate and/or non-viable *Fusobacterium nucleatum* or *Porphyromonas gingivalis* (MOI 1:100) to determine the molecular effects of α-lipoate on oral epithelial responses to periodontopathic bacteria. Immunocytochemical analyses demonstrated that NF-κB was maximally activated at 1 h of exposure to both bacteria and that *F. nucleatum* stimulated a significantly greater number of NF-κB nuclear translocations than *P. gingivalis*. Gene expression analyses showed that cytokine transcripts regulated by the NF-κB pathway (TNF-α, IL-1β, IL-8 & GM-CSF) were up-regulated following 24 h of exposure to both bacteria. Pre-incubation of H400 cells with 0.5mM α-lipoate (24h) decreased NF-κB translocation and cytokine gene expression stimulated by both bacteria. ELISA analyses of supernatants for IL-8 and IL-1β confirmed the effects of a-lipoate (TNF-α & GM-CSF were not detectable at 24h). Toll-like receptor (-2, -4 & -9) transcripts were variously affected by bacterial stimulation and α-lipoate treatment. TLR-2 expression was unaffected whereas TLR-4 was upregulated by bacterial challenge and further enhanced by α-lipoate. TLR-9 expression was unaffected by *F. nucleatum* and downregulated by *P. gingivalis*, but α-lipoate pre-treatment upregulated expression irrespective of bacterial stimulation. These data highlight the potential therapeutic role of α-lipoate in modifying local inflammatory-immune responses for preventing development/progression of periodontitis, a disease where hyper-inflammation underlies susceptibility.

Rat brain antioxidant defences decrease after cocaine administration

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Introduction: Oxidative stress has been involved in the pathogenesis of several diseases, and may be a pathogenic mechanism underlying many psychiatric disorders, as the brain has comparatively greater vulnerability to oxidative damage. Cocaine is a highly addictive drug that is associated with serious complications in the CNS. The neurobiological changes that accompany drug addiction are not well understood, and the cellular and molecular mechanisms of these alterations may involve oxidative injury. Therefore it is of interest to study what happen to different brain structures in terms of oxidative stress, after long term administration of cocaine. Material and methods: For this purpose rats were injected daily 15 mg/kg of cocaine during 3 weeks. After sacrifice and brain dissection, the concentration of the reduced (GSH) and oxidized (GSSG) forms of glutathione, as well as glutathione peroxidase (GPx) activity were determined in the homogenate from hippocampus, diencephalon, cerebellum, frontal cortex and striatum. Results and discussion: GSH/GSSG ratio and GPx activity in all structures were decreased, with the exception of frontal cortex, thus indicating that oxidative stress is, somehow involved in the cocaines's central mechanism of action. Therefore the compensatory mechanisms to scavenge reactive oxygen species, reported by other groups, occurring in response to cocaine abuse are not effective after 3 weeks of cocaine administration. Finally, our results show that frontal cortex seems to be more resistant to oxidative stress being in agreement with others, which have previously described that low doses of cocaine does not induce a decrease of catalase levels in frontal cortex, in contrast to what happen to other structures such as striatum.

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Structure-activity relationships in the radical-scavenging reaction of polyphenolic flavones

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The radical-scavenging activity of naturally occurring polyphenols has attracted considerable interest with regard to prevention of diseases induced by oxidative stress. However, very little is known about the structure-activity relationships in the radical-scavenging reactions of polyphenolic antioxidants. In this study, we examined the dynamics of the scavenging reaction of cumylperoxyl radical by four polyphenolic flavones, quercetin, luteolin, kaempferol, and apigenin, in propionitrile (EtCN) at 203 K by using the electron spin resonance (ESR) technique.

The decay of the ESR signal of cumylperoxyl radical at g = 2.0153 generated by photoirradiation of an EtCN solution of cumene, di-*tert*-butylperoxide, and O2 at 203 K was observed in the presence of quercetin, luteolin, or kaempferol, suggesting that cumylperoxyl radical can be efficiently scavenged by these polyphenolic flavones. Apigenin shows no scavenging activity. The second- order rate constants of the scavenging reactions are determined from the decay of the ESR signal intensity of cumylperoxyl radical, as 1.7 x 10^3 $M^{-1}s^{-1}$ (quercetin), 5.6 x 10^2 $M^{-1}s^{-1}$ (kaempferol), and 2.6 x 10^2 $M^{-1}s^{-1}$ (luteolin). These results suggest that not only the OH group at C-3f position on the B ring but also that at C-3 position on the C ring plays an essential role in the radical-scavenging reaction. The effect of a metal ion on the rates will also be discussed to elucidate the radical-scavenging mechanism by the polyphenolic flavones.

Inhibitory effect of antioxidants on hydroxyl radicals generated from Methylguanidine: an ESR study

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Methylguanidine is known as not only a nephrotoxin but also as a neurotoxin. Guanidino compounds, such as guanidinobutyric acid [1] and methylguanidine [2]. After intracisternal injection these compounds induce convulsion in experimental animals, e.g., rats and rabbits. Sustained increased levels of Methylguanidine were found in the brain of experimental models of epilepsy: amygrala- or hippocampus-kindled rats. Also it was demonstrated that Methylguanidine itself generates hydroxyl radicals in an in vitro study [3]. In this study, we examined the radical scavenging activities of ascorbate, Trolox, EPC-K1 (α-tocopheryl-L-ascorbate-2-*o*-phosphate diester), and GSH for hydroxyl radicals generated from Methylguanidine using an ESR technique with a spin trap. These compounds showed potent inhibitory effects on hydroxyl radical generation from methylguanidine.

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Na^+/H^+-Exchanger-1 gene deficiency counteracts high glucose-induced oxidative-nitrosative stress and experimental diabetic neuropathy

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Na^+/H^+-exchanger-1 (NHE-1) overexpression and activation increase cytosolic pH with concomitant activation of glucose transport and enzymes of the upper part of glycolysis. In diabetes, this may lead to diversion of excessive glycolytic flux to several pathways leading to oxidative stress and diabetic complications. This study was aimed at evaluating the role of NHE-1 in high glucose-induced oxidative stress in Schwann cells (SC) and development of experimental peripheral diabetic neuropathy (PDN). PDN was compared in control (C) and STZ-diabetic (STZ-D) wild-type and NHE-1+/- mice. In in vitro experiments, human SC were transfected with NHE-1 siRNA, and cultured in 5.5 mM or 30 mM glucose. NHE-1, nitrosylated protein and 4-hydroxynonenal adduct expressions were assessed by Western blot analyses. Individual SC NHE-1 activity and basal intracellular pH were measured by pH imaging technique and signals from 20-25 HC were averaged. STZ-D NHE-1+/- mice were completely protected from SNCV deficit, and partially protected from MNCV deficit and sensory neuropathy that were clearly manifest in STZ-D wild-type mice. 24-h exposure of human SC to 30 mM glucose resulted in 0.31 unit increase in intracellular pH, and a 43% increase in NHE-1 activity. NHE-1 siRNA transfection of human SC resulted in ~65% reduction in NHE-1 expression, and alleviated high glucose-induced intracellular oxidative-nitrosative stress. In conclusion, NHE-1 plays an important role in high glucose-induced oxidative-nitrosative stress in SC and PDN.

Modulation of mitochondrial complex I activity and nNOS expression in experimental model of Parkinson's disease – Neuroprotective effect of curcuminoids

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Parkinson's disease (PD) is a multifactorial, age related neurodegenerative disorder, characterized by the dopaminergic neuronal degeneration in substantia nigra. Growing evidences suggest that mitochondrial damage, particularly Complex I is directly involved in the pathogenesis of PD. Progress in the search for effective therapeutic strategies that can halt this degenerative process remains limited. Curcuminoids, the polyphenols of Curcuma longa (L.) is an established antioxidant against different reactive oxygen and nitrogen species. The present study investigates the neuroprotective and anti-Parkinsonian effects of curcuminoids in MPTP model of PD. Male C57BL/6 mice were injected once with MPTP injection (15 mg/kg body weight) for six consecutive days. Prior to MPTP injection the mice were treated with curcuminoids (25 mg/kg body weight) for one week and continued further for three months after induction. We observed a clear loss of mitochondrial Complex I activity on MPTP induction which was protected against in curcuminoids treated animals. We also found elevation in ROS and ONOO- level with concomitant reduction in total glutathione content in the MPTP treated mice. Curcuminoids administration significantly decreases the ROS and $ONOO^-$- generation and prevented the glutathione depletion. Our study reflects that increased $ONOO^-$ level is due to overexpression of nNOS, which was prevented on curcuminoids treatment. In addition, the present study demonstrates pronounced restoration of TH-IR fibres in substantia nigra. In conclusion, the results suggest that curcuminoids exerts neuroprotective effect by accelerating antioxidant defense mechanism,preventing Complex I activity degradation and exhibiting nNOS inhibiting property. Together, curcuminoids may act as a promising agent for the treatment of PD.

Lipopolysaccharide induces adaptive response and enhances PC12 cell tolerance

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It is known that lipopolysaccharide (LPS) induces oxidative stress and inflammatory cytokines, which leads to apoptosis including neuronal cells. Past studies indicated that some toxic stimuli at sublethal concentrations can induce adaptive response and enhance cell tolerance. We focused on adaptive cytoprotection induced by LPS especially in THP-1 which is human macrophage and PC12 cells which has been used as a model of neuronal cell. After treating THP-1 and PC12 cells with LPS at sublethal concentration, we found that they developed resistance to subsequent toxicity induced by 13S-hydroperoxy-9Z, 11E-octadecadienoic acid (13(S)-HpODE) and 5-amino-3-(4-morpholinyl)-1,2,3-oxadiazolium (SIN-1). In order to determine the underling molecular mechanisms responsible for an adaptive response induced by LPS, we studied the changes in antioxidant system such as glutathione and thioredoxin reductase. However, none of the studies showed any significant changes. Therefore, gene expression profiles in LPS-treated PC12 cells were determined by using oligonucleotide microarrays. It showed a striking increase of glutathione S-transferase (GST) gene expression in LPS-treated cells. We confirmed the fact that GST plays an important role in adaptive response by down regulating GST expression using RNA interference and GST inhibitor. Also, we found that nuclear factor-erythroid 2 p45-related factor 2 (Nrf2) plays a key role in the adaptive response through GST regulation. The current study proved the ability of cytoprotection afforded by LPS with emphasis on its potential to modulate Nrf2-mediated GST induction.

Anti-oxidant activity of methanolic extracts of *Achyranthes aspera*

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Free radicals are implicated for more than 100 diseases including diabetes mellitus, arthritis, cancer, ageing, etc. In treatment of theses diseases, antioxidant therapy as gained an utmost importance. Current research is now directed towards finding naturally occurring antioxidant of plant origin. In Indian system of medicine Achyranthes aspera is an important medicinal plant and its leaves, seeds and roots have been used in various ailments. To understand the mechanisms of pharmacological actions, the in vitro antioxidant activity of methanolic extract of leaf, seed and root of Achyranthes aspera was investigated for activity of scavenging superoxide anion radicals, nitric oxide radical, and DPPH free radicals. In all the testing, a significant correlation existed between concentrations of the extracts and percentage inhibition of free radicals. The percentage inhibition in all three antioxidant models, methanolic leaf extract of Achyranthes aspera showed very potent activity then rest of the extracts. The antioxidant property may be related to the antioxidant vitamins, phenolic acids and micronutrients present in the extracts. These results clearly indicate that Achyranthes aspera is effective against free radical mediated diseases. KEYWORDS - Antioxidant activity, Achyranthes aspera, Super oxide, Nitric oxide, DPPH free radicals.

Hepatoprotective activity of *Moringa oleifera Lam*, fruits and leaves on isolated rat hepatocytes

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Study was conducted to test the hypothesis that Moringa oleifera Lam has hepatoprotective activity. For this purpose Study was designed using different concentration of crude aqueous and ethanol (alcoholic) extract of moringa oleifera Lam. fruit and leaves to investigate protection against CCl_4 (10 mM) induced hepatocytes injury of rats in vitro and compared with standard silymarin concentrations of 0.001, 0.005 and 0.01 mg/ml. Results showed that extract was effective in the reducing CCl_4 induced enhanced activities of GPT, GOT, lipid peroxidation and increasing % viability. As per results obtained from the study it is evident that low concentration of alcoholic extracts has significant hepatoprotective activity in concentration of 0.01 mg/ml while that of aqueous extract at minimum 0.1 mg/ml and these results shows better activity for leaves than fruit. This shows hepatocellular damage caused by CCl_4 and its recovery by pretreatment with the crude extracts. Hepatoprotective activity could be related to the free radical scavenging properties of various components present in the extract which is evident from the antioxidant level measurement. In conclusion it is suggested that Moringa oleifera Lam. leaves might be considered as a potential source of natural hepatoprotective agent.

Oxidative reactive secondary sequence atherosclerosis-lipid model for determining free-radical antioxidant activity

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Oxygen is vital to brain tissue Life in order to remove electrons and acid produced during mitochondria energy synthesis. The Blood Brain Barrier ensures that nonpolar molecules like oxygen readily pass through and prevents damaging electrons and acids from accumulating in the brain. However, electrons not picked up by oxygen during brain cell metabolism can participate in free-radical chain lengthening of lipids in blood vessels. Vessel occlusion at every level then interferes with oxygen delivery at some distant point. In order to test a functional biologic model for atherosclerosis, lipids (90:10 oleic:linoleic) and acrolein (C_3H_4O), a common toxic lipid breakdown product, were mixed at different lipid concentrations ranging from 50-100% in a plastic container. A redox couple was then added that consisted of 4wt% benzoyl peroxide and 4wt% organometallic cobalt naphthenate. After a few months several hardened organic substances were produced as biologic evidence for lipid chain lengthening by reactive secondary sequence across repeated alkene double bonds with the vinyl group from acrolein. In addition, a hardened crystalline material was produced as a manifestation of lipid peroxidative crosslinking by oxygen from the atmosphere at the lipid:acrolein surface interface with the plastic reaction container. Complete disappearance of the white peroxide granules showed a remarkable exponential relationship rather than linear for acrolein concentrations from 50-20%. For acrolein concentrations at 10 and 0%, not all of the benzoyl peroxide was consumed. The biologic model is thus ready for easy use to test the effectiveness of antioxidants in removing free radicals to prevent lipid chain lengthening and also molecular oxygen lipid peroxidation to ensure proper blood oxygen delivery for the brain.

Neuroprotective effect of *Hippopheae rhamnoides* on hyperhomocysteinemia-mediated oxidative stress in aged brain

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Hyperhomocysteinemia is an important risk factor in the pathogenesis of age related neurodegenerative disorder. Abundant evidences suggest that plasma homocysteine (Hcy) level increases with age, which is neurotoxic. Elevated oxidative stress is one of the important mechanism for Hcy neurotoxicity. Seabuckthorn (*Hippopheae rhamnoides*) berries are rich source of vitamins, folic acid and potent antioxidant. The present study aims to investigate the neuroprotective effect of seabuckthorn berries on hyperhomocysteinemia and oxidative stress markers in C57BL/6 aged mice. Hydro alcoholic extract of the drug (180 mg/kg) was orally administered to mice (8 and 15 months) for three months. A significant increase in Hcy level and decrease in folic acid content was found in aged brain. Further, marked elevation in ROS levels with concomitant reduction in GPx activity and MnSOD expression were observed in aged brain. Drug treatment reverses the above changes back to normal level. In addition, GSH/GSSG ratio was maintained towards reduced state on drug administration, which was earlier found to be shifted to the oxidized state in aged brain. Drug pretreatment prevents all the age-associated changes. These results indicate that seabuckthorn berries maintains the normal homocysteine levels by supplementing vitamins and folic acid, which declines with age and accelerates antioxidant defense mechanism suppressed by hyperhomocysteinemia. Thus, Hippopheae rhamnoides exerts neuroprotective activity and its use as a supplement for amelioration of age related neurodegenerative disorder needs further research.

Gestational diabetes and oxidative stress

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The main objective of this research work was to evaluate if there was oxidative damage and the level of that damage in gestational diabetes. In the present study thirty healthy women and thirty women with gestational diabetes were analyzed in the three trimesters of pregnancy regarding their levels of oxidative stress. The mean values of MDA for the healthy group of women were 0.73 ± 0.40 mcmolar in the first trimester, 0.68 ± 0.57 mcmolar in the second one and 0.76 ± 0.43 mcmolar in the third trimester. The group of women with gestational diabetes had all the values with higher levels and with significative differences in the first and in the second trimester ($p<0.019$ and $p<0.028$ respectively), namely with values of 1.04 ± 0.59 mcmolar in the first trimester, 1.06 ± 0.83 mcmolar in the second one and 0.82 ± 0.61 mcmolar in the third trimester. The mean values of GPx for the healthy women were 70.63 ± 16.87 nanomoles/mg prot x min in the first trimester, 66.62 ± 21.33 nanomoles/mg prot x min in the second and 68.13± 16.97 in the third trimester. The mean values of GPx for the diabetic women were always lower (43.92 ± 21.75 nanomoles/mg prot x min , 63.04 ± 38.26 nanomoles/mg prot x min , y 65.11 ± 33.45 nanomoles/mg prot x min respectively) with significative differences in the first trimester ($p < 0.001$). In this observational and longitudinal study in gestating women, the damage due to oxidative stress was present before the biochemical detection of the gestational diabetes, and its treatment (diet and exercise, without medication) lowered it. The GPx activity in women with gestational diabetes increases during the gestational period, possibly as an attempt of compensation at the high levels of oxidative stress.

Regulation of H_2O_2-induced necrosis by protein kinase C and AMP-activated kinase signaling in primary cultured hepatocytes

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In this study we examined whether necrosis induced by H_2O_2 is regulated by signaling pathways in primary hepatocytes. A detailed time course revealed that H_2O_2 given to hepatocytes is consumed within minutes, but hepatocytes undergo necrosis several hours later. Thus H_2O_2 treatment induces a "lag phase" where signaling changes occur including Protein Kinase C (PKC) activation, Akt (PKB) down regulation, activation of c-Jun kinase (JNK) and down regulation of AMP activated kinase (AMPK). Investigation of various inhibitors demonstrated that PKC inhibitors were effective in reducing necrosis caused by H_2O_2 (~ 80%). PKC inhibitor treatment decreased PKC activity, but surprisingly also upregulated Akt and AMPK, suggesting various PKC isoforms negatively regulates Akt and AMPK. Work with various PKC inhibitors suggests classical PKC, likely PKC-b, negatively regulates Akt in primary hepatocytes. However, Akt does not appear to play a significant role in H_2O_2-induced necrosis, since PKC inhibitor treatment protected hepatocytes from H_2O_2 even when Akt was inhibited. On the other hand, Compound C, a selective AMPK inhibitor, abrogated the protective effect of PKC inhibitors against necrosis induced by H_2O_2, suggesting that much of the protective effect of PKC inhibition was mediated through upregulation of AMPK. Work with PKC inhibitors suggest that atypical PKC downregulates AMPK in response to H_2O_2. Knocking down PKC-α using antisense also slightly protected (~22%) against H_2O_2. Taken together our data demonstrate that modulation of signaling pathways involving AMPK and PKC can determine H_2O_2-induced necrosis, suggesting a signaling "program" is important in mediating H_2O_2-induced necrosis in primary hepatocytes.

A multivitamin–mineral preparation with guaraná positively effects cognitive performance and reduces mental fatigue during sustained mental demand

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This double-blind, randomised, placebo-controlled, parallel group study examined the effects of a multivitamin–mineral supplement with guaraná (Berocca Boost Performance®) on aspects of cognitive performance and self-reported mental fatigue during sustained, effortful mental processing. The acute effects of a single dose of either the vitamin/mineral/guaraná or placebo in an effervescent drink were assessed in 129 healthy adults (18–24 years). The Cognitive Demand Battery (CDB) is a 10-min battery comprising of Serial Threes subtraction (2 min), Serial Sevens subtraction (2 min), Rapid Visual Information Processing [RVIP] (5 min) and a 'Mental Fatigue' visual analogue scale. Following a baseline assessment, participants consumed their treatment and thirty minutes later completed the CDB six times consecutively. The most notable findings were that the active treatment was associated with increased speed and accuracy in the performance of the RVIP (a measure of vigilance and working memory) throughout the 60 min of testing. The active treatment was also associated with reduced ratings of subjective mental fatigue significantly so during the later, more fatiguing, repetitions. This research supports the previous findings of the psychoactive properties of guaraná and is not dissimilar to other findings from our groups following cocoa polyphenol administration. The study provides for the first time evidence in humans that a multivitamin–mineral preparation containing guaraná can improve cognitive performance and reduce mental fatigue associated with sustained mental effort.

Reactive oxygen species mediated DNA damage and its modulation by EDTA and ethanol

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Reactive oxygen species (ROS) interact with components of a living cell and introduce an array of changes in them. The hydroxyl radicals ($^{\cdot}OH$) are believed to contribute maximally towards cellular damage. The cellular concentration of ROS, especially $^{\cdot}OH$, correlates well with mutagenesis, carcinogenesis and ageing as well as other pathophysiological conditions. Therefore, proper handling of the cellular ROS is critical to well being and survival of a life form. Interestingly, the major source of generation of such ROS is also internal - the inherent metabolic processes generate significant ROS load in a cellular system. Other exogenous sources such as chemicals, ionizing radiation, etc. also generate ROS in the living system. For obvious reasons, understanding the ROS inflicted DNA damage and its processing have always dominated investigations to understand its biological implications. This study used a plasmid DNA construct, pMTa4, *in vitro* and *in vivo* to understand the kinetics of SSB and DSB induced by two free radical generating systems (FRGS), namely, Fenton and Haber-Weiss reagents. Two different interventions were used to nullify the ROS burden on pMTa4 DNA - a metal ion chelator, ethylenediaminetetraacetic acid (EDTA) and a free radical scavenger, ethanol. EDTA (10 mM) as well as ethanol (400 mM) completely abolished induction of DSB by both FRGS. However, EDTA was unable to abolish the dose-dependent induction of SSB by either FRGS unlike ethanol, which completely prevented further induction of SSB. Results suggest that EDTA and ethanol mediated protections to induction of strand breakage in plasmid DNA were qualitatively and quantitatively different for free radical and γ-radiation. The presentation shall focus on the mechanistic aspect of these observations.

Synergistic interaction of phenolic acid from *Euphorbia hirta* and their protective role in oxidative injury to protein

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Natural phenolic acids are of great interest in oxidative stress due to their antioxidant role in protection to biomolecules. The phenolic acid constituents from aqueous extract of Euphorbia hirta leaves were fractionated on sephadex LH-20 column chromatography. The phenolic acid constituents were ascertained using GC-MS and HPLC technique. To ascertain their efficacy to scavenge free radical and antioxidant potential, DPPH free radical scavenging assay and FRAP assay were performed. To determine the modulatory interaction with reference bovine serum albumin in the presence of metal catalysed oxidation system (MCO), we employed spectrophotometric protein carbonyl estimation assay using DNPH (Dinitrophenyl hydrazine), SDS-PAGE and western blot technique to detect protein carbonyl using anti-DNP primary antibody. Phenolic acid enriched fractions are identified and mainly benzoic acid and cinnamic acid structure based phenols were present. The fractions possessed strong antioxidant activity as revealed from above free radical scavenging assays. Phenolic acids showed synergistic interaction with BSA and their antioxidant activity was found to be enhanced after incubation with BSA to extent of 25.12 percent. The protective role against MCO catalysed oxidative damage to BSA was also evaluated and results substantiated the significant contribution of phenolic acids with an effective modulatory concentration of EC_{50} 150.41 µg/ml to inhibit oxidative damage to BSA. The natural antioxidant as phenolic acid constituents from E. hirta could be concluded as promising free radical scavenger and showed satisfactory synergistic interaction with BSA. The results convincingly showed effectiveness in protection against oxidative injury to BSA.

Role of JNK translocation to mitochondria leading to inhibition of mitochondria bioenergetics in acetaminophen-induced liver injury

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Previously, we demonstrated JNK plays a central role in acetaminophen (APAP)-induced liver injury. In this study, we examine the mechanism involved in activating JNK and explore the downstream targets of JNK important in promoting APAP-induced liver injury. JNK inhibitor (SP600125) significantly protected against APAP-induced liver injury in mice. Increase mitochondria-derived reactive oxygen species (ROS) were implicated in APAP-induced JNK activation based on the following: 1) mitochondrial GSH depletion (maximal at 2 h) caused increased H_2O_2 release from mitochondria which preceded JNK activation (peak at 4 h); 2) treatment of hepatocytes with H_2O_2 or inhibitors (e.g. antimycin) that cause increased H_2O_2 release from mitochondria activated JNK. Mitochondria were observed to be important downstream targets of JNK based on the following: 1) JNK translocated to mitochondria following activation; 2) JNK inhibitor treatment partially protected against a decline in respiration caused by APAP; and 3) addition of purified active JNK to mitochondria isolated from mice treated with APAP plus JNK inhibitor (mitochondria with severe GSH depletion) directly inhibited respiration. Cyclosporin A blocked the inhibitor effect of JNK on mitochondria respiration, suggesting JNK was directly inducing MPT in redox altered mitochondria. Addition of JNK to mitochondria isolated from control mice did not affect respiration. Our results suggests that APAP-induced liver injury involves JNK activation, due to increased ROS generated by GSH depleted mitochondria, and translocation of activated JNK to mitochondria where JNK induces MPT and inhibits mitochondria bioenergetics.

The effects of the antioxidant Pycnogenol© on cognitive performance, serum lipids, endocrinological and oxidative stress biomarkers in an elderly population

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The study examined the effects of the antioxidant flavonoid Pycnogenol (PYC) on a range of cognitive and biochemical measures in healthy aged individuals. The study employed a double-blind, placebo controlled, matched pairs design, with 101 elderly participants (60-85 yrs) consuming a daily dose of 150mg of PYC for a three-month treatment period. Participants were assessed at baseline, then at one, two and three months of the treatment. The Control (placebo) and PYC groups were matched by age, sex, body mass index (BMI), micronutrient intake and intelligence. The cognitive tasks comprised measures of attention, working memory, episodic memory and psycho-motor performance. The biological measures comprised levels of clinical hepatic enzymes, serum lipid profile, human growth hormone and lipid peroxidation products. Statistically significant interactions were found for memory based cognitive variables and lipid peroxidation products, with the PYC group displaying improved working memory and decreased concentrations of f2-isoprostanes relative to the control group.

Evaluation of multi-parameter biomarker set for oxidative stress assessment

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Considerable evidence indicating that oxidative stress is unhealthy and leads to serious disease is compelling. Increased oxidative stress generally describes a condition in which cellular antioxidant defenses are inadequate to completely inactivate the reactive oxygen species (ROS) and reactive nitrogen species (RNS). This can occur because of excessive production of ROS/RNS and/or loss of antioxidant defenses. The major consequences of oxidative stress are damage to DNA/RNAs, lipids, and proteins, which can severely compromise cell health and viability, ultimately leading to the development of multiple acute and chronic human diseases, such as cancer, heart disease, diabetes, and neurodegeneration, as well as with the aging process itself. We believe that research on the role of oxidative damage in the aging process and age-related diseases and the testing of interventions to prevent oxidative damage cannot be on a firm scientific basis without first having a reliable set of tools for measuring oxidative stress levels. Therefore, our goal has been to develop sensitive and reliable methods to measure the oxidative stress status (OSS). We have developed LC-MS/MS methods to quantify DNA/RNA damage products (8-hydroxy-2'-deoxy guanosine, 8-hydroxy-guanosine), lipid peroxidation products (8-iso-prostaglandin F2α, iPF2α-VI, and 2,3-dinor-8-iso-prostaglandin F2α, 8,12-iso-iPF2α-VI, 2,3-dinor-5,6-dihydro-8-iso-prostaglandin F2α) and protein damage products (dityrosine, nitrotyrosine, and chlorotyrosine) in human samples. In this presentation, we will discuss multi-parameter biomarker set for oxidative stress assessment and role of oxidative stress in age and other age-related diseases.

Drosophila as a genetic platform for analysis of Keap1/Nrf2 signaling

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The transcription factor Nrf2 and its inhibitor Keap1 comprise the major system of antioxidant and detoxification defense. Keap1/Nrf2 signaling protects cells and organisms against the detrimental effects of oxidative stress, and abates its consequences, including aging-associated diseases like neurodegeneration, chronic inflammation, and cancer. Nrf2 is a prominent target for drug discovery, and Nrf2-activating agents like oltipraz are in clinical trials for cancer chemoprevention. Nrf2 belongs to the capâ€™nâ€™collar family of leucine zipper transcription factors, which is named after the cnc gene locus of the fruitfly, Drosophila melanogaster. We have demonstrated that the Drosophila cnc locus encodes a functional Nrf2 homologue, and that a keap1 orthologue is also present in the fruitfly. Oxidants, electrophiles, and cancer chemopreventive drugs like oltipraz potently stimulate Drosophila Nrf2 activity. Similarly to its function in vertebrates, Keap1/Nrf2 signaling in the fruitfly induces antioxidant and detoxification responses, and confers increased tolerance to oxidative stress. Importantly, keap1 loss-of-function mutations extend the lifespan of male Drosophila, supporting a novel role for Nrf2 signaling in the regulation of longevity. We will present our genetic approaches using the fruitfly system to identify and characterize novel genes that modulate Keap1/Nrf2 signaling.

Phytoestrogen of young coconut juice reduced hyperlipidemia and lipid peroxidadation in over weight rats

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Phytoestrogen consumption has been shown to reduce risk of cardiovascular disease and neurodegenerative disease, such as Alzheimer's disease. The aim of this study was to investigate the effect of phytoestrogen of extract young coconut Juice (YCJ), Cocos nucifer (Arecaceae), in hypolipemic and lipid peroxidation activity in over weight rats and was given by oral route at dose of 5 mL/kg BW/day for 4 weeks. Serum cholesterol, triglyceride, LDL-cholesterol levels were significantly reduced, but no effected on the serum level of HDL-cholesterol. It is also significantly reduced malondialdehyde (MDA), the lipid peroxidation products. The consumption of young coconut juice improved the lipidemic profile and reduced lipid peroxidation, suggesting that young coconut juice might contribute to a reduction of cardiovascular risk and neurodegenerative disease.

Neuroprotective effects of sulforaphane in an *in vitro* model of Parkinson's disease

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There is growing interest in dietary strategies aimed at counteracting oxidative stress-induced neuronal death associated with Parkinsons disease (PD). Isothiocyanates (ITCs), present in cruciferous vegetables, are known as cancer chemopreventive agents and strong inducers of phase II detoxification enzymes. Among the various ITCs, sulforaphane (SUL) has recently gained attention as a potential neuroprotective agent. In this study, we investigated the mechanism basis of the neuroprotective potential of SUL in a neuronal cell model of PD. In particular, we demonstrated that treatment of human neuronal-like SH-SY5Y cells with SUL prevents the oxidative damage and neuronal death induced by 6-hydroxidopamine (6-OHDA), a specific neurotoxin. In parallel, we found a potent indirect antioxidant activity of SUL on neuronal cells that could be ascribed to increased GSH levels and phase II enzyme activities, such as glutathione-S-transferase, glutathione reductase and NADPH-quinone. Interestingly, SUL also showed an ability to rescue the neuronal death induced by 6-OHDA through the activation of neuronal survival pathways such as PI3K/AKT and MEK/ERK. Taken together, these findings suggest that SUL may have a positive impact on PD to retard or reverse the accelerated rate of neuronal degeneration.

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Cyanidin 3-O-glucopyranoside protects Sh-SY5Y cells against amyloid-β peptide-induced toxicity

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The Amyloid-β (Aβ) peptide is the major component of senile plaques that are one of the hallmarks of Alzheimer's disease (AD). Accumulating evidence shows that different monomeric, oligomeric and fibrillar species of Aβ peptides affect neuronal viability, having a causal role in the development of AD. Recently, many studies have reported anti-amyloidogenic and neuroprotective effects of dietary antioxidant components. In this study, we evaluated the protective effects of cyanidin 3-O-glucoside (Cy-3G), an anthocyanin present in red fruits and vegetables, against Aβ peptide-induced toxicity in terms of mitochondrial dysfunction and apoptosis in human neuronal-like SH-SY5Y cells. In our experimental approach, Cy-3G neuroprotective effects were evaluated by adding it to the SH-SY5Y cells before, during and after the treatment with monomeric Aβ25-35, oligomeric and fibrillar Aβ1-42 peptides, known to be involved in AD. In particular, SH-SY5Y cells treated with Cy-3G prevented the mitochondrial dysfunction induced by Aβ25-35, but not Aβ1-42 peptides. By contrast, the concomitant treatment with Cy-3G inhibited both neurotoxicity parameters induced by all Ab peptides used. Further, Cy-3G showed an ability to rescue the mitochondrial dysfunction and apoptosis induced by monomeric Aβ25-35 and oligomeric Aβ1-42, but not fibrillar Aβ1-42. Interestingly, under aggregating conditions in vitro, Cy-3G also inhibited the fibrillar Aβ1-42 formation. These preliminary results encourage further research in animal models of AD to explore the potential neuroprotective activity of Cy-3G and other anthocyanins.

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Flow-cytometric analysis of mitochondria heterogeneity: methodological development

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Mitochondria mass changes with dynamic balance of fusion and fission during mitochondria function and injury. Fragmentation of mitochondria apparently accompanies cytochrome c release and apoptosis. On the other hand, elongated giant mitochondria in chronic alcohol abuse are a consistent common feature and seemingly viewed as resting mitochondria. In fact, a methodology, simultaneously analyzing the single mitochondria mass and biochemical correlation in statistically reliable mitochondria population, is necessary for the study of mitochondria biology. We developed a flow-cytometric method using Mito-Tracker dye to detect the changes in isolated mitochondria population in acetaminophen (APAP) and alcohol induced liver injury. The liver mitochondria population shifted to right, indicating an increasing enlarged population with time after APAP injection. This change in individual mitochondria mass correlated with decreased RCR (respiratory chain rate) measured polarographically with a Clark type electrode(Hanstech, UK). We compared the sensitivity and accessibility of flow-cytometric determination of individual mitochondria mass change and visual counting of isolated mitochondria in confocal microscopy images. Counting in 2 dimensional visual images showed no significant difference in mitochondria population between control and chronic alcohol fed mice. In contrast, flow-cytometric analysis indicated an increased population of giant mitochondria in chronic alcohol fed mice. Flow-cytometric analysis is a fast, reproducible and sensitive method to determine 3 dimensional mass of mitochondria. This study is an initial development of methodology and we are working on determination of functional changes of individual mitochondria.

Keap1 recruits Nrf2 to Cul3-based E_3 ligase by two-site substrate recognition: a hinge and latch mechanism for oxidative/electrophilic stress response

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Nrf2 is the regulator of the oxidative/electrophilic stress response. The E_3 ligase adaptor Keap1 recognizes Nrf2 through its conserved ETGE and DLG motifs and constitutive repression of Nrf2 is maintained by Keap1-mediated proteasomal degradation via a two-site substrate recognition mechanism. Recognition of Nrf2 by Keap1 targets the multiple lysine residues in the Neh2 domain of Nrf2 to be ubiquitinated by the Cul3-based E_3 ligase. The ETGE and DLG motifs of Nrf2 are recruited by the Keap1 homodimer in a similar fashion but with different binding affinities. Further studies have deciphered that different electrostatic potentials primarily define the ETGE and DLG motifs as a hinge and latch that senses the oxidative/electrophilic stress for cellular defensive response.

What makes the peroxidatic cysteine in CysGPx reactive?

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Cysteine homologues of glutathione peroxidases (GPxs) have more recently surprised with rate constants for the oxidation of the peroxidatic cysteine (C_P), k'_{+1} of > 10^6 $M^{-1}s^{-1}$. This observation conflicts with the common view that the pronounced reactivity just results from dissociation of CP enforced by hydrogen bonding within the catalytic triad composed of Cys/Sec, Gln and Trp, because even a fully dissociated cysteine (or any other low MW thiol) does not react with H_2O_2 with a rate constant > 5 x 10^3 $M^{-1}s^{-1}$. Sequence homology of > 400 sequences, modeling of the *Drosophila melanogaster* GPx (*Dm*GPx) as a paradigm, and site-directed mutagenesis revealed a pivotal role of Asn136 as a fourth essential component of the active site. Mutational substitution of Asn136 by His, Ala or Asp results in a decline of specific activity by 97, 98 and 100%, respectively. In steady-state kinetic analysis of the muteins k'_{+1} is decreased by two- to three orders of magnitude, while the reductive steps characterized by k'_{+2} are less affected. Accordingly, MS/MS analysis shows that in Asn136 mutants the peroxidatic Cys45 (C_P) stays largely reduced also in the presence of a hydroperoxide, while in the wild-type enzyme it is oxidized forming a disulfide with the resolving Cys (C_R). Computational calculation of pK_a values from wild-type and mutant *Dm* GPxs indicates that the residues facing the catalytic thiol, Asn136, Gln80 and, to a lesser extent Trp135, contribute to the dissociation of the thiol group, Asn136 being most relevant. It has, however, to be postulated that Asn 136 and Gln 80 activate C_P not only by enforcing thiol dissociation but also by proton shuttling leading to polarization of the substrate's peroxo bond, protonation of leaving groups and product stabilization. In conclusion, the catalytic site of GPxs has to be redrawn as a tetrad, wherein "carboxamide catalysis" is most relevant to the extraordinary catalytic efficiency of GPxs.

High-resolution detection for ·OH decay by natural antioxidants: A spin trapping ESR study

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We established a high-resolution detection system for ·OH decay in the presence of antioxidants. A spin trapping agent CYPMPO enables ·OH visible by ESR of spin adducts. ·OH were produced successfully by the UV illumination upon H_2O_2 without using Fenton reaction. ·OH were trapped by a spin trapping agent CYPMPO immediately after production of ·OH by UV illumination. We confirmed the existence of ·OH through ESR spectroscopy. Upon application of antioxidant onto the ·OH-containing system, we found the decay reaction of ·OH by the various antioxidants such as Blueberry, Raspberry and Haskap. The decay process was detected in the snap-shot manner at every 5 s interval, so that detail of the reaction kinetics was revealed, for the first time. The reaction scheme was the pseudo-first order and the time constant was found to be c.a. 0.2 s^{-1}. The high resolution detection of ·OH and their decay processes may allow unveiling the mechanisms for biomedical antioxidation.

Mitochondrial function and oxidative stress in sepsis: Effect of lipoic acid

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Experimental sepsis occurs with mitochondrial dysfunction and massive increases in nitric oxide production, as part of its pathogenic mechanism. Previous studies showed that oxidative stress is implicated in the tissue damage observed in sepsis, so an antioxidant therapy would be beneficial. Consequently, lipoic acid (an antioxidant with redox properties) was chosen. Sprague-Dowley female rats, 180 g, were injected with 10 mg/kg LPS (lipopolisaccharide) and the assays were carried out after 6 h of treatment. When necessary, lipoic acid (dose: 100 mg/kg) was co-injected with LPS. The study was focused on muscle tissues. A 5.8 fold increase in organ chemiluminescence (CL) was observed for skeletal muscle (control: 7 ± 1 cps/ cm^2 ;treated: 41 ± 3 cps / cm^2) while liver CL remained unchanged. Oxygen consumption by tissue slices showed a 30-40% increase in heart and diaphragm of septic animals, while the state 3 oxygen uptake in muscle mitochondria was observed to be 40% decreased. NO production was also increased, 90% in diaphragm mitochondria (control: 0.69 ± 0.05 nmol NO/min mg prot) and 30% in heart mitochondria (control: 0.77 ± 0.08 nmol NO/min mg prot). The activity of the mitochondrial complexes I, II and IV, were also measured. Lipoic acid treatment in septic rats: a) decreased skeletal muscle CL; b) returned the mitochondrial NO production to control values. The available data suggest that the oxidative stress and the impairment of mitochondrial function constitute the basis of the molecular mechanism of organ dysfunction in sepsis, that can be partially reverted by lipoic acid.

Oxidative stress indicators and heavy metal levels in Bottlenose dolphin (*Tursiops truncatus*) tissues from Bahía de La Paz, México

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High concentrations of heavy metals contribute to cellular damage by promoting an increase in ROS production and oxidative stress. It has been suggested that marine mammals have a constitutively high antioxidant status that allows them to cope with routine increases in dive-derived ROS production. In order to elucidate the potential relationship between heavy metal levels and oxidative stress in dolphin tissues, we measured superoxide radical (O2•–) production, lipid peroxidation (TBARS), superoxide dismutase (Mn-SOD and Cu,Zn-SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, as well as heavy metal levels (Cd, Cu, Fe, Ni, Zn) in heart, liver, lung and muscle tissue samples from bottlenose dolphins (*Tursipos truncatus*) stranded in Bahía de La Paz, México. O2•– production was higher ($p<0.05$) in lung than in heart, liver or muscle. CAT and GPx activities were higher ($p<0.05$) in liver than in the other tissues. Cu levels were higher ($p<0.05$) in heart and liver than in muscle or lung, and Zn levels were higher ($p<0.05$) in liver than heart or lung. TBARS, Cd, Fe and Ni levels, Mn-SOD and Cu,Zn-SOD activities did not differ between tissues. Results show that despite differences found in O2•– production, as well as in Cu and Zn levels, oxidative damage is not statistically different between dolphin tissues. The latter could be related to an enhanced antioxidant system in this species which is probably associated to its adaptations to the diving lifestyle.

(-)-Epicatechin and related procyanidins modulate intracellular calcium and prevented oxidation in Jurkat T cells

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We investigated the effects of (-)-epicatechin (EC), its oligomers, dimer B2 (B2), and trimer C1 (C1), on calcium mobilization and cell oxidation. Jurkat T cells were subjected to a calcium mobilization challenge by replacing Na^+ with K^+ (K) in the incubation media. A 200 % increase in intracellular calcium concentration ($[Ca]_i$) was observed, and that effect was prevented by the presence of inhibitors of calcium mobilization. The preincubation of the cells in the presence of EC, B2 or C1 prevented K-mediated increase in $[Ca]_i$. IC_{50} were 10, 24, and 197 nM for EC, B2 and C1, respectively. Cell membrane depolarization was affected by K, but neither inhibitors of calcium mobilization, EC, B2, or C1 modified the increase in membrane potential. A 98 % increase in cell oxidants was observed after cell exposure to K. This increase was prevented by the inhibition of calcium mobilization, NADPH oxidase, and protein kinase C, as well as by 10 nM EC, 10 nM B2, or 100 nM C1. In addition, EC and B2 (100 nM) significantly inhibited the activation of the $[Ca]_i$-regulated transcription factor NFAT. These results indicate that EC and related oligomers, assayed at physiologically achievable concentrations, can modulate $[Ca]_i$ and then prevent cell oxidation and other calcium-regulated events.

Activation of PKR by mild impairment of oxidative metabolism in neurons

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Thiamine (vitamin B_1) deficiency (TD) causes mild and chronic impairment of oxidative metabolism and induces neuronal death in specific brain regions. The mechanisms underlying TD-induced cell death, however, remain unclear. The double-stranded RNA (dsRNA)-activated protein kinase (PKR) has been well known for its anti-viral function. Upon activation by viral infection or dsRNA, PKR phosphorylates its substrate, the a-subunit of eukaryotic translation factor-2 ($eIF_2\alpha$), leading to inhibition of translation. In response to various cellular stresses, PKR can also be stimulated by its protein activators, PACT, or its mouse homologue RAX. We demonstrated that TD in mice induced phosphorylation of PKR at Thr^{446} and Thr^{451} and phosphorylation of eIF_2a at Ser^{51} in the cerebellum and the thalamus. TD caused phosphorylation of PKR and $eIF_2\alpha$ as well as nuclear translocation of PKR in primary cultures of cerebellar granule neurons (CGNs). PKR phosphorylation is necessary for its nuclear translocation because TD failed to induce nuclear translocation of a T446A/T451A PKR mutant. Both PKR inhibitor and dominant-negative PKR mutant protected CGNs against TD-induced cell death. TD promoted the association between RAX and PKR. Antioxidant vitamin E dramatically decreased the RAX/PKR association and ameliorated TD-induced cell death. Our results indicate that TD-induced neuronal death is at least partially mediated by the activation of PKR.

Tryptophan-derived kynurenine is a novel endothelium-derived relaxing factor

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Control of blood vessel tone is central to vascular homeostasis. Conditions of oxidative stress are associated with loss of vascular homeostasis, in part due to endothelial dysfunction caused by overproduction of superoxide. Here, we report kynurenine (Kyn) as a novel endothelium-dependent vascular relaxing factor formed from the amino acid tryptophan by the superoxide-consuming enzyme indoleamine 2,3-dioxygenase (IDO) during systemic inflammation. Infection of mice with Plasmodium berghei ANKA, and LPS-induced endotoxemia caused endothelial expression of IDO, resulting in decreased plasma tryptophan, increased Kyn, and systemic hypotension. Pharmacological inhibition of IDO increased blood pressure in malaria-infected and in endotoxemic mice, but not in the respective control animals or in mice deficient in IDO or interferon, which is required for IDO induction. Tryptophan dilated pre-constricted porcine coronary arteries only if active IDO and an intact endothelium were present. Kyn dose-dependently decreased blood pressure in spontaneously hypertensive rats, inhibited contraction of arteries, and relaxed pre-constricted rings in an endothelium-independent manner. Arterial relaxation by Kyn was mediated by activation of the adenylate and soluble guanylate cyclase pathways. Strikingly, Kyn activated heme-free soluble guanylate cyclase more effectively than the heme-containing enzyme that becomes refractory to activation by NO under conditions of oxidative stress.

Oxidative stress challenge of peripheral blood lymphocytes provides a non-invasive predictor of brain DNA damage

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Increasing evidence suggests the availability of micronutrients significantly influences cognitive and behavioral function. Because oxidative stress contributes to brain aging and other neuropathologies, we tested the hypothesis that a non-invasive predictor of antioxidant status could identify individuals with the highest level of brain DNA damage. This hypothesis was tested in a population of 69 elderly male beagle dogs after they had completed a 7 month randomized feeding trial to achieve the broad range of dietary selenium status observed in U.S. men. For each dog, the alkaline Comet assay was used to directly compare brain DNA damage with the sensitivity of peripheral blood lymphocytes (PBLs) to oxidative stress-induced DNA damage. Using stepwise logistic regression, the sensitivity of PBLs to oxidative stress challenge with hydrogen peroxide (H2O2) predicted dogs in the highest tertile of brain (cerebral cortex) DNA damage. Dogs with PBLs highly sensitive to H2O2 were 5 times [95% confidence interval (95% CI), 1.3-19.5] more likely to have high brain DNA damage than those in the H2O2-resistant group. This risk stratification was observed in multivariate analysis that considered other factors that might influence DNA damage, such as age, toenail selenium concentration, and serum testosterone concentration. These findings support the notion that oxidative stress contributes to brain DNA damage, and that oxidative stress challenge of blood cells may identify those individuals who might benefit from nutrition-based neuroprotective interventions.

Fisetin inhibits poly(ADP-ribose)polymerase (PARP)-1 and the release of inflammatory cytokines

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The flavonol fisetin (3,7,3',4'-tetrahydroxyflavone) exhibits antioxidant, anti-inflammatory, neuroprotective and antiproliferative effects and is suggested as a potentially useful therapeutic agent against free-radical mediated diseases. Its precise mechanism of action is not fully elucidated yet, although latest in vitro data indicate that fisetin modulates MAPK activities and NF-kB mediated pathways. Recently, the nuclear enzyme PARP-1 has been identified as co-activator of the NF-kB-mediated transcription of inflammatory cytokines. Due to its involvement in the pathophysiology of acute and chronic inflammation, PARP-1 emerges as an interesting target in the treatment of diseases like COPD or diabetes mellitus. We investigated the anti-inflammatory effects of fisetin in more detail. Fisetin (100 mM) strongly inhibited purified human PARP-1 activity in vitro. It attenuated N-methyl-N'-nitro-N-nitrosoguanidine-induced decrease in NAD+ levels and inhibited dose-dependently PAR polymer formation in human lung epithelial cells. Lung tissue of mice orally pre-treated with fisetin (100 mM/kg bodyweight/day) over 4 days revealed significantly lower gene expression levels of proinflammatory cytokines after intratracheal LPS (20 mg/mouse) installation. In whole blood of diabetic and COPD patients fisetin (10 mM) significantly reduced LPS-induced (1 ng/ml) release of TNF-a and IL-6. Our preclinical results support the anti-inflammatory activity of fisetin and make it to a promising candidate for a further clinical evaluation.

Blue native PAGE analysis of dihydrolipoamide dehydrogenase

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Dihydrolipoamide dehydrogenase (DLDH) is the E3 subunit shared by mitochondrial alpha-keto acid dehydrogenase complexes. The decrease in the activity of DLDH-associated complexes in brain has been shown to represent a common element in several age-related neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. Based on the property that DLDH can act as a diaphorase catalyzing in vitro NADH-dependent reduction of electron-accepting molecules such as nitroblue tetrazolium (NBT), we have developed a gel-based method for histochemical staining and quantification of mitochondrial DLDH diaphorase activity using blue native PAGE (BN-PAGE). Rat brain mitochondrial extracts, as the source of DLDH, were used for the assay development. Mitochondrial proteins were resolved by non-gradient BN-PAGE (9%), which was followed by diaphorase activity staining using NADH as the electron donor and NBT as the electron acceptor. It was shown that activity staining of DLDH diaphorase was both protein amount- and time-dependent. Moreover, this in-gel activity-staining method was demonstrated to be in good agreement with the conventional spectrophotometric method that measures DLDH dehydrogenase activity using dihydrolipoamide as the substrate. When applied to evaluate the effects of thiol-reactive reagents on DLDH diaphorase activity, the method demonstrates that DLDH diaphorase activity can be determined without having to remove the thiol-reactive reagents that may otherwise interfere with spectrophotometric measurement of DLDH dehydrogenase activity. The gel-based method can also be used as a means to isolate mitochondrial DLDH that is to be analyzed by mass spectral techniques in studying DLDH post-translational modifications.

Mitochondria play a pivotal role in estrogen-induced neuroprotection against Alzheimer's disease

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We have investigated the role of mitochondria in neuroprotection induced by estrogen. We have previously shown that the neuroprotective effects of 17β-estradiol (E2) are dependent upon intact mitochondrial function and many of the signaling cascades induced by E2 treatment converge upon the mitochondria. In the current study we sought to characterize the role of mitochondria in the pathogenesis of Alzheimer's disease (AD) as well as their role in E2 induced neuroprotection against AD in a triple transgenic AD (3xTg-AD) mouse model. Compared to the age-matched NonTg group, aged 3xTg-AD mice showed compromised mitochondrial functions, including decreased pyruvate dehydrogenase protein level and enzyme activity, decreased complex IV subunit IV (COXIV) activity, decreased mitochondrial respiration. In addition to the deficits in energy production, there is a higher amyloid beta level in the mitochondria of 3xTg-AD mice with a correlating increase in the level of amyloid β alcohol dehydrogenase (ABAD), which leads to a higher oxidative stress in the 3xTg-AD mice as manifested in the increase in hydrogen peroxide production and higher lipid peroxidation. Further, we demonstrated that E2 treatment increased the protein expression and activity of PDH and COXIV. A 24hr subcutaneous treatment of E2 at 125 m g/kg significantly decreases the mitochondrial amyloid and ABAD level. Meanwhile, E2 treatment increases MnSOD activities. These coordinated regulation of mitochondrial proteins contributed to an enhanced mitochondrial function, including higher energy production and lower oxidative stress. Results of this study suggest that mitochondria play a pivotal role in estrogen-induced neuroprotection against AD related pathology.

Nitric oxide modulation of glutathionylation: Implications for neurodegeneration

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The role of NO in neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease, stems from the excessive production and labile nature of NO. The mechanisms of NO redox signaling through protein post-translational modifications is ill defined in the neurodegenerative model. S-nitrosylation of proteins has emerged as a well-characterized mechanism through which NO reversibly regulates cell function. However, S-nitrosylation of proteins can act as an intermediary step leading to S-glutathionylation. There is little evidence concerning the regulation of S-glutathionylation of proteins by NO and which proteins are glutathionylated in neurons during nitrosative stress. Hence, the impetus for this study was to investigate the role of NO modulated S-glutathionylation of proteins in neuronal cell death. Our results indicate that acute exposure of neurons to NO, mirroring neuroinflammation, led to the formation of S-glutathoinylated proteins. Exposure to exogenous NO also resulted in an alteration of cellular redox status through the formation of intracellular GSNO and GSSG. Increasing concentrations of GSNO and GSSG formation as a consequence of NO exposure correlated with S-glutathionylation of proteins. Glutathonylation of GAPDH, a key glycolytic enzyme, led to significant inhibition of its activity. These observations delineate a potential mechanism through which increased NO production, a key event in neurodegeneration, could potentially lead to protein S-glutathionylation and neuronal dysfunction seen in Friedreich's ataxia and Alzheimer's disease.

Aging- and caloric restriction-induced change in glutaredoxin and thioredoxin activity in brain mitochondria

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Mitochondria are the major source of reactive oxygen species (ROS) that affect the redox status of the cell and, as such the regulation of cell signaling and transcription. Dysfunctional mitochondria generate higher amounts of oxidants that cause damage to mitochondrial components, initiate degradative processes, and contribute significantly to the aging process. Glutaredoxin and thioredoxin are small thiol-disulfide oxidoreductases that regulate target proteins by thio/disulfide exchanges and glutathiolation/deglutathiolation, respectively. These two systems play important roles in combating against oxidative stress. However, to date, there has been no extensive study undertaken to investigate their role in aging process. In this study, aging and caloric restriction (CR) rat models are used to find out the upstream regulation and downstream consequences of Grx and Trx functions under oxidative stress. CR feeding is an experimental protocol to delay aging by slowing of accrual of oxidative stress. Our preliminary data showed that aging and CR diet induces a change of activities of these redox components in rat brain mitochondria, and these changes may influence metabolic control and redox signaling through post-translational modification of mitochondrial proteins. Information obtained in this study could lead to a better understanding of the energy-redox interactions and redox homoeostasis control.

Hydroxyoctadecadienoic acid and oxidatively modified peroxiredoxin in blood of Alzheimer's disease patients: the potential biomarkers

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The central nervous system is particularly vulnerable to oxidative damage and it has been suggested that oxidative stress may play an important role in the pathogenesis of Alzheimer's disease (AD). In fact, many studies have shown the increased levels of oxidation-associated metabolites and decline of antioxidant level in biological fluids of AD patients. Although oxidative stress has been implicated in the early stage of this disease, its detailed pathogenesis and therapeutic targets remain unknown. The diagnosis, especially at the early stage, is important. In this decade, F_2-isoprostanes from arachidonates and neuroprostanes from docosahexanoates have been proposed as biomarkers. Although these markers are formed by a free radical-mediated oxidation, the yields from the parent lipids are minimal. Compared to these markers, hydroperoxy octadecadienoates (HPODE) from linoleates are yielded by much simpler mechanisms from more abundant parent lipids in vivo. We have proposed the method in which both free and ester forms of hydroperoxides and ketones as well as hydroxides of linoleic acid are measured as total hydroxyoctadecadienoic acid (tHODE). In the present study, the levels of tHODE and oxidatively modified peroxiredoxin (oxPrx)-2 and oxPrx-6 in plasma and/or erythrocytes were determined by a GC-MS apparatus and by 2-dimensional electrophoresis, respectively. It was found that these levels in AD patients were significantly higher than those in the healthy controls. Furthermore, the tHODE levels increased with increasing clinical dementia rating. Interestingly, vascular dementia patients could be distinguished from the correlation of plasma and erythrocyte levels of tHODE or from that of tHODE with oxPrx in erythrocytes. These data suggest that oxidative stress is indeed involved in AD and that tHODE and oxPrx are potential biomarkers for the diagnosis of AD.

Effect of L-NAME, L-arginine, and sildenafil on mitochondrial nitric oxide metabolism in rat heart adaptation to high altitude

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This work studies heart mtNOS activity and expression in the adaptive response to high altitude at Cerro de Pasco (Perú, 4,340m, PO_2=12.2kPa) and the effect of pharmacological treatments on that adaptation. Rats were mantained at sea level (SL) and high altitude (HA); 2 groups were control (SL-C, HA-C), 2 groups were treated with L-NAME (8.3 mg/kg.day, SL-N and HA-N), 2 with L-arginine (106 mg/kg.day; SL-A and HA-A), and 2 with sildenafil (50 mg/kg.day; SL-S and HA-S). Animals were sacrificed at 7, 14, 21, 28, 42 and 84 days. HA animals showed right ventricle hypertrophy at 42 and 84 days. Heart mtNOS activity was 70% higher in HA than in SL animals and neither of the treatments suppressed this response. The increase in mtNOS activity was accompanied by an increase of 60% in mtNOS expression. Heart mtNOS activity was about 150% higher in SL-N than in SL-C, suggesting that sustained inhibition of mtNOS results in an up-regulation of this enzyme. The combination of HA and L-NAME produced a faster enhancement (10%) in mitochondrial NO production than HA itself. Conversely, sildenafil treatment impaired mtNOS activity response to HA (20%). All the groups maintained at HA showed an hyperbolic response of hematocrit as a function of time of exposure. The mean values of hematocrit and mtNOS activity from HA rats correlated linearly ($R2=0.82$, $P>0.05$). We conclude that mtNOS is a substantial source of cardiac NO and constitutes a factor in the adaptive response to sustained heart hypoxia, susceptible to be modified by pharmacological treatments.

Protective effect of green tea polyphenols against 6-OHDA-induced apoptosis through ROS-NO pathway in Parkinson's disease models

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We here investigate the protective mechanisms of GTP in Parkinsons disease (PD) model *in vitro* and *in vivo*. In the model of 6-OHDA induced SH-SY5Y cells apoptosis, GTP attenuated 6-OHDA-induced early apoptosis, prevented the decrease in mitochondrial membrane potential, suppressed accumulation of ROS and of intracellular Ca^{2+}. GTP also counteracted 6-OHDA-induced NO increase and over-expression of nNOS and iNOS, and decreased the level of protein bound 3-Nitrotyrosine. In addition, GTP inhibited the auto-oxidation of 6-OHDA and scavenged ROS in a dose and time dependent manner. In the 6-OHDA induced animal model, injection of 6-OHDA caused typical PD phenotypes and damage in the brain and treatment of GTP protected the animals against the toxicity of 6-OHDA. More neurons survived and less cells suffered apoptosis in the substantia nigra of GTP treated animal brain. Results showed that treatment of the animals with GTP decreased ROS and NO production, TBARS content, nitrite/nitrate concentration, and protein bound 3-NT and increased the antioxidant ability in brain homogenate of midbrain and striatum in a concentration and time dependent manner. NOS participated in the neuron death induced by 6-OHDA and the treatment with GTP could decrease the protein level of nNOS and iNOS in midbrain and striatum. These results suggest that oral administration of GTP protected the rat against PD induced by 6-OHDA through ROS-NO pathway in vivo. Our results show that the protective effects of GTP in PD model are mediated, at least in part, by ROS-NO pathway.

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