Literaturrecherche nitrosativer Stress

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1: Cancer Res 2008 Sep;68(18):7457-65

Multimodal Control of Cdc25A by Nitrosative Stress.

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Cdc25A propels cell cycle progression, is overexpressed in numerous human cancers, and possesses oncogenic and antiapoptotic activities. Reactive oxygen species, such as hydrogen peroxide, regulate Cdc25A, but the physiologic and pathologic effects of nitric oxide (*NO) and *NO-derived reactive species are not well defined. Herein, we report novel independent mechanisms governing Cdc25A in response to nitrosative insult. We observed direct and rapid inhibition of Cdc25A phosphatase activity after in vitro treatment with the low molecular mass cell-permeable S-nitrosothiol S-nitrosocysteine ethyl ester (SNCEE). In addition, treatment of cancer cells with SNCEE induced nitrosative stress and decreased Cdc25A protein levels in a time-dependent and concentration-dependent manner. Similarly, iNOS-derived *NO was sufficient to suppress Cdc25A expression, consistent with its role in mediating nitrosative stress. Whereas a decrease in Cdc25A half-life was not observed in response to SNCEE, we found the translational regulator eukaryotic initiation factor 2alpha (eIF2alpha) was hyperphosphorylated and total protein translation was decreased with kinetics consistent with Cdc25A loss. Inhibition of eIF2alpha decreased Cdc25A levels, supporting the hypothesis that SNCEE suppressed Cdc25A translation through inhibition of eIF2alpha. Nitrosative stress decreased the Cdc25A-bound fraction of apoptosis signal-regulating kinase-1 (ASK-1) and sensitized cells to apoptosis induced by the ASK-1-activating chemotherapeutic cis-diaminedichloroplatinum (II), suggesting that nitrosative stress-induced suppression of Cdc25A primed cells for ASK-1-dependent apoptosis. Together these data reveal novel *NO-dependent enzymatic and translational mechanisms controlling Cdc25A, and implicate Cdc25A as a mediator of *NO-dependent apoptotic signaling. [Cancer Res 2008;68(18):7457-65].

2: Am J Pathol 2008 Aug;

Enhanced Nitrosative Stress during Trypanosoma cruzi Infection Causes Nitrotyrosine Modification of Host Proteins. Implications in Chagas' Disease.

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Oxidative/nitrosative stress may be important in the pathology of Chagas' disease. Experimental animals infected by Trypanosoma cruzi showed an early rise in myocardial and peripheral protein-3-nitrotyrosine (3NT) and protein-carbonyl formation that persisted during the chronic stage of disease. In comparison, experimental chronic ethanol-induced cardiomyopathy was slow to develop and presented with a moderate increase in oxidative stress and minimal to no nitrosative stress after long-term alcohol feeding of animals. The oxidative stress in both chagasic animals and animals with ethanol-induced cardiomyopathy correlated with the persistence of reactive oxygen species-producing inflammatory intermediates. Protein-3NT formation in T. cruzi-infected animals was associated with enhanced nitric oxide expression (inferred by nitrite/nitrate levels) and myeloperoxidase activity, suggesting that both peroxynitrite- and myeloperoxidase-mediated pathways contribute to increased protein nitration in Chagas' disease. We used one- and two-dimensional gel electrophoresis and Western blot analysis to identify disease-specific plasma proteins that were 3NT-modified in T. cruzi-infected animals. Nitrated protein spots (56 in total) were sequenced by matrix-assisted laser desorption ionization/time of flight mass spectrometry and liquid chromatography-tandem mass spectrometry and identified by a homology search of public databases. Clustering of 3NT-modified proteins according to their functional characteristics revealed that the nitration of immunoglobulins, apolipoprotein isoforms, and other proteins might perturb their functions and be important in the pathology of Chagas' disease. We also showed that nitrated peptides derived from titin and alpha-actin were released into the plasma of patients with Chagas' disease. Such modified proteins may be useful biomarkers of Chagas' disease.

3: Cell Biol Int 2008 Aug;

Dynamic determination of Ox-LDL-induced oxidative/nitrosative stress in single macrophage by using fluorescent probes.

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Increased oxidative/nitrosative stress, resulting from generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) appears to play an important role in the inflammatory responses to atherosclerosis. By using MitoTracker Orange CM-H(2)TMRos, CM-H(2)DCFDA (DCF-DA), Dihydrorhodamine 123 (DHR123), DAF-FM, Dihydroethidium (DHE) and JC-1 alone or in all combinations of red and green probes, the present study was designed to monitor the ROS and RNS generation in acute exposure of single monocyte U937-derived macrophage to oxidized low density lipoprotein (Ox-LDL). Acute Ox-LDL (100mug/ml) treatment increased time-dependently

production of intracellular nitric oxide (NO), superoxide (O(2)(-)), hydrogen peroxide (H(2)O(2)) and peroxynitrite (ONOO(-)), and decreased mitochondrial membrane potential (Deltapsi) in single cell. Pretreatment of aminoguanidine (an inhibitor of inducible nitric oxide synthase (iNOS), 10muM) and vitamin C (an antioxidant agent, 100muM) for 2h, reduced significantly the Ox-LDL-induced increase of NO and O(2)(-), and vitamin C completely inhibited increase of intracellular NO and O(2)(-). In contrast to aminoguanidine, Vitamin C pretreatment significantly prevented Ox-LDL-induced overproduction of NO and O(2)(-) ($P<0.01$), indicating that antioxidant may be more effective in therapeutic application than iNOS inhibitor in dysfunction of ROS/RNS. By demonstrating a complex imbalance of ROS/RNS via fluorescent probes in acute exposure of single cell to Ox-LDL, oxidative/nitrosative stress might be more detected in the early atherosclerotic lesions.

4: Eukaryot Cell 2008 Aug;

Protein Kinase C1 (PKC1) is essential for protection against both oxidative and nitrosative stress, cell integrity, and normal manifestation of virulence factors in the pathogenic fungus Cryptococcus neoformans.

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Cell wall integrity is crucial for fungal growth, survival and pathogenesis. Responses to environmental stresses are mediated by the highly conserved Pkc1 protein and its downstream components. In this study, we demonstrate that both oxidative and nitrosative stress activate the PKC1 cell integrity pathway in wild type cells, as measured by phosphorylation of Mpk1, the terminal protein in the PKC1 phosphorylation cascade. Furthermore, deletion of PKC1 shows that this gene is essential for defense against both oxidative and nitrosative stress; however, other genes involved directly in the PKC1 pathway are dispensable for protection against these stresses. This suggests that Pkc1 may have multiple and alternative functions other than activating the MAP kinase cascade from a "top down" approach. Deletion of PKC1 also causes osmotic instability, temperature sensitivity, severe sensitivity to cell wall inhibiting agents, and alterations in capsule and melanin. Furthermore, the vital cell wall components chitin and its deacetylated form chitosan appear to be mislocalized in a pkc1Delta strain, although this mutant contains wild type levels of both of these polymers. These data indicate that loss of Pkc1 has pleiotropic effects because it is central to many functions either dependent or independent of PKC1 pathway activation. Notably, this is the first time that Pkc1 has been implicated in protection against nitrosative stress in any organism.

5: J Biol Chem 2008 Aug;

Oxygen- and NssR-dependent globin expression and enhanced iron acquisition in the response of campylobacter to nitrosative stress.

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Pathogenic bacteria experience nitrosative stress from NO generated in the host and from nitrosating species such as S-nitrosoglutathione (GSNO). The foodborne pathogen Campylobacter jejuni responds by activating gene expression from a small regulon under the control of the NO-sensitive regulator, NssR. Here, we describe the full extent of the GSNO response using transcriptomic and proteomic analysis of batch- and chemostat-cultured C. jejuni. In addition to the NssR regulon, which includes two hemoglobins (Cgb and Ctb), we identify more than 90 other up-regulated genes, notably those encoding heat shock proteins and proteins involved in oxidative stress tolerance and iron metabolism/transport. Up-regulation of a subset of these genes, including cgb, is also elicited by NO-releasing compounds. Mutation of the iron-responsive regulator Fur results in insensitivity of growth to NO, suggesting that derepression of iron-regulated genes and augmentation of iron acquisition is a physiological response to nitrosative damage. We describe the effect of oxygen availability on nitrosative stress tolerance: cells cultured at higher rates of oxygen diffusion have elevated levels of hemoglobins, are more resistant to inhibition by NO of both growth and respiration, and consume NO more rapidly. The oxygen response is mediated by NssR. Thus, in addition to NO detoxification catalyzed by the hemoglobins Cgb and possibly Ctb, C. jejuni mounts an extensive stress response. We suggest that inhibition of respiration by NO may increase availability of oxygen for Cgb synthesis and function.

6: Am J Physiol Renal Physiol 2008 Apr;

Chronic L-Arginine Administration Increases Oxidative and Nitrosative Stress in Rat Hyperoxaluric Kidneys and Excessive Crystal deposition.

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Hyperoxaluric kidneys show impaired diuretic response to acute infusion of L-arginine. In this study, we studied the effects of chronic L-arginine supplementation on CaOx crystal formation in hyperoxaluric rat kidneys. Eight groups were tested: the control group received drinking water, L group received L-arginine (0.6%), LN group received N(G)-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg), L+LN group received L-arginine plus L-NAME, HP group received hydroxyl-L-proline (HP, 5%) mixed with chow to induce hyperoxaluria, L+HP group received HP plus L-arginine, HP+LN group, and L+HP+LN group. The duration was 42 days and each group had n=8. Urinary biochemistry and renal CaOx amounts were measured, as well as renal expressions of NOS isoforms and NAD(P)H oxidase. The distribution of iNOS, NAD(P)H oxidase, ED1-positive cells, and nitrotyrosine were examined by immuno-histochemical and immuno-fluorescence studies, while superoxide production from the kidneys was examined by fluorescence spectrometric assay. Compared to the HP group, the L+HP group had excessive CaOx crystal accumulation and enhanced eNOS, iNOS, and NAD(P)H oxidase protein expression in the kidney. Urinary excretion of nitrotyrosine was markedly increased. Increased superoxide formation in the L+HP kidney was derived from NAD(P)H oxidase and uncoupled eNOS, and increased nitrotyrosine formation might derive from iNOS and ED1-positive cells which gathered around the CaOx crystals. L-NAME co-treatment (L+HP+LN group) reduced renal oxidative, nitrosative stress, and tubular damage which were induced by

L+HP. The results showed that chronic L-arginine treatment to the hyperoxaluric kidney with many CaOx crystal deposition may have a toxic effect by enhancing intra-renal oxidative and nitrosative stress. Key words: hyperoxaluria, nephrolithiasis, NAD(P)H oxidase, nitric oxide synthase.

7: Lab Invest 2008 Jun;

The mitochondrial permeability transition, and oxidative and nitrosative stress in the mechanism of copper toxicity in cultured neurons and astrocytes.

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Copper is an essential element and an integral component of various enzymes. However, excess copper is neurotoxic and has been implicated in the pathogenesis of Wilson's disease, Alzheimer's disease, prion conditions, and other disorders. Although mechanisms of copper neurotoxicity are not fully understood, copper is known to cause oxidative stress and mitochondrial dysfunction. As oxidative stress is an important factor in the induction of the mitochondrial permeability transition (mPT), we determined whether mPT plays a role in copper-induced neural cell injury. Cultured astrocytes and neurons were treated with 20 muM copper and mPT was measured by changes in the cyclosporin A (CsA)-sensitive inner mitochondrial membrane potential (DeltaPsim), employing the potentiometric dye TMRE. In astrocytes, copper caused a 36% decrease in the DeltaPsim at 12 h, which decreased further to 48% by 24 h and remained at that level for at least 72 h. Cobalt quenching of calcein fluorescence as a measure of mPT similarly displayed a 45% decrease at 24 h. Pretreatment with antioxidants significantly blocked the copper-induced mPT by 48-75%. Copper (24 h) also caused a 30% reduction in ATP in astrocytes, which was completely blocked by CsA. Copper caused death (42%) in astrocytes by 48 h, which was reduced by antioxidants (35-60%) and CsA (41%). In contrast to astrocytes, copper did not induce mPT in neurons. Instead, it caused early and extensive death with a concomitant reduction (63%) in ATP by 14 h. Neuronal death was prevented by antioxidants and nitric oxide synthase inhibitors but not by CsA. Copper increased protein tyrosine nitration in both astrocytes and neurons. These studies indicate that mPT, and oxidative and nitrosative stress represent major factors in copper-induced toxicity in astrocytes, whereas oxidative and nitrosative stress appears to play a major role in neuronal injury.Laboratory Investigation advance online publication, 30 June 2008; doi:10.1038/labinvest.2008.49.

9: Free Radic Biol Med 2008 Apr;

Effects of oxidative and nitrosative stress in brain on p53 proapoptotic protein in amnestic mild cognitive impairment and Alzheimer disease.

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Many studies reported that oxidative and nitrosative stress might be important for the pathogenesis of Alzheimer's disease (AD) beginning with arguably the earliest stage of AD, i.e., as mild cognitive impairment (MCI). p53 is a proapoptotic protein that plays an important role in neuronal death, a process involved in many neurodegenerative disorders. Moreover, p53 plays a key role in the oxidative stress-dependent apoptosis. We demonstrated previously that p53 levels in brain were significantly higher in MCI and AD IPL (inferior parietal lobule) compared to control brains. In addition, we showed that in AD IPL, but not in MCI, HNE, a lipid peroxidation product, was significantly bound to p53 protein. In this report, we studied by means of immunoprecipitation analysis, the levels of markers of protein oxidation, 3-nitrotyrosine (3-NT) and protein carbonyls, in p53 in a specific region of the cerebral cortex, namely the inferior parietal lobule, in MCI and AD compared to control brains. The focus of these studies was to measure the oxidation and nitration status of this important proapoptotic protein, consistent with the hypothesis that oxidative modification of p53 could be involved in the neuronal loss observed in neurodegenerative conditions.

11: Int J Mol Med 2008 Jun;21(6):667-76

Aldose reductase inhibitor fidarestat counteracts diabetes-associated cataract formation, retinal oxidative-nitrosative stress, glial activation, and apoptosis.

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This study was aimed at evaluating the potent and specific aldose reductase inhibitor fidarestat, on diabetes-associated cataract formation, and retinal oxidative-nitrosative stress, glial activation, and apoptosis. Control and streptozotocin-diabetic rats were treated with or without fidarestat (16 mg kg-1d-1) for 10 weeks after an initial 2-week period without treatment. Lens changes were evaluated by indirect ophthalmoscopy and portable slit lamp. Nitrotyrosine, poly(ADP-ribose), and glial fibrillary acidic protein expression were assessed by immunohistochemistry. The rate of apoptosis was quantified in flat-mounted retinas by TUNEL assay with immunoperoxidase staining. To dissect the effects of high glucose exposure in retinal microvascular cells, primary bovine retinal pericytes and endothelial cells were cultured in 5 or 30 mM glucose, with or without fidarestat (10 muM) for 3-14 days. Apoptosis was assessed by TUNEL assay, nitrotyrosine and poly(ADP-ribose) by immunocytochemistry, and Bax and Bcl-2 expression by Western blot analyses. Fidarestat treatment prevented diabetic cataract formation and counteracted retinal nitrosative stress, and poly(ADP-ribose) polymerase activation, as well as glial activation. The number of TUNEL-positive nuclei (mean +/- SEM) was increased approximately 4-fold in diabetic rats vs. controls (207+/-33 vs. 49+/-4, $p<0.01$), and this increase was partially prevented by fidarestat (106+/-34, $p<0.05$ vs. untreated diabetic group). The apoptotic cell number increased with the prolongation of exposure of both pericytes and endothelial cells to high glucose levels. Fidarestat counteracted nitrotyrosine and poly(ADP-ribose) accumulation and apoptosis in both cell types. Antiapoptotic effect of fidarestat in high glucose-exposed retinal pericytes was not associated with the inhibition of Bax or increase in Bcl-2 expression. In conclusion, the findings, i) support an important role for aldose reductase in

diabetes-associated cataract formation, and retinal oxidative-nitrosative stress, glial activation, and apoptosis, and ii) provide a rationale for the development of aldose reductase inhibitors, and, in particular, fidarestat, for the prevention and treatment of diabetic ocular complications.

12: Mol Immunol 2008 Apr;

Differential contribution of neutrophilic granulocytes and macrophages to nitrosative stress in a host-parasite animal model.

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Tyrosine nitration is a hallmark for nitrosative stress caused by the release of reactive oxygen and nitrogen species by activated macrophages and neutrophilic granulocytes at sites of inflammation and infection. In the first part of the study, we used an informative host-parasite animal model to describe the differential contribution of macrophages and neutrophilic granulocytes to in vivo tissue nitration. To this purpose common carp (Cyprinus carpio) were infected with the extracellular blood parasite Trypanoplasma borreli (Kinetoplastida). After infection, serum nitrite levels significantly increased concurrently to the upregulation of inducible nitric oxide synthase (iNOS) gene expression. Tyrosine nitration, as measured by immunohistochemistry using an anti-nitrotyrosine antibody, dramatically increased in tissues from parasite-infected fish, demonstrating that elevated NO production during T. borreli infection coincides with nitrosative stress in immunologically active tissues. The combined use of an anti-nitrotyrosine antibody with a panel of monoclonal antibodies specific for several carp leukocytes, revealed that fish neutrophilic granulocytes strongly contribute to in vivo tissue nitration most likely through both, a peroxynitrite- and an MPO-mediated mechanism. Conversely, fish macrophages, by restricting the presence of radicals and enzymes to their intraphagosomal compartment, contribute to a much lesser extent to in vivo tissue nitration. In the second part of the study, we examined the effects of nitrosative stress on the parasite itself. Peroxynitrite, but not NO donor substances, exerted strong cytotoxicity on the parasite in vitro. In vivo, however, nitration of T. borreli was limited if not absent despite the presence of parasites in highly nitrated tissue areas. Further, we investigated parasite susceptibility to the human anti-trypanosome drug Melarsoprol (Arsobal), which directly interferes with the parasite-specific trypanothione anti-oxidant system. Arsobal treatment strongly decreased T. borreli viability both, in vitro and in vivo. All together, our data suggest an evolutionary conservation in modern bony fish of the function of neutrophilic granulocytes and macrophages in the nitration process and support the common carp as a suitable animal model for investigations on nitrosative stress in host-parasite interactions. The potential of T. borreli to serve as an alternative tool for pharmacological studies on human anti-trypanosome drugs is discussed.

13: Neuro Endocrinol Lett 2008 Jun;29(3)

An IgM-mediated immune response directed against nitro-bovine serum albumin (nitro-BSA) in chronic fatigue syndrome (CFS) and major depression: Evidence that nitrosative stress is another factor underpinning the comorbidity betwee.

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It has been shown that chronic fatigue syndrome (CFS) and major depression (MDD) are accompanied by signs of oxidative stress and by a decreased antioxidant status. The aim of the present study was to examine whether CFS and MDD are accompanied by an IgM-mediated immune response directed against nitro-serum bovine albumin (BSA), which is a neoepitope of BSA formed by damage caused by nitrosative stress. Toward this end, we examined serum IgM antibodies to nitro-BSA in 13 patients with CFS, 14 subjects with partial CFS, 16 patients with MDD and 11 normal controls. We found that the prevalence and mean values for the serum IgM levels directed against nitro-BSA were significantly greater in patients with partial CFS, CFS and MDD than in normal controls, and significantly greater in CFS than in those with partial CFS and MDD. We found significant and positive correlations between serum IgM levels directed against nitro-BSA and symptoms of the FibroFatigue scale, i.e. aches and pain and muscular tension. There was also a strong positive correlation between serum IgM titers directed against nitro-BSA and an index of increased gut permeability ("leaky gut"), i.e. serum IgM and IgA directed against LPS of different gram-negative enterobacteria. The abovementioned results indicate that both CFS and MDD are accompanied by a) an increased gut permeability which has allowed an exaggerated passage of BSA through a compromised epithelial barrier; b) increased nitrosative stress which has induced damage to BSA; and c) an IgM-mediated immune response which is directed against the nitro-BSA neoepitopes. Nitrosative stress is one of the factors underpinning the comorbidity and clinical overlap between CFS and MDD.

14: Neurology 2007 Dec;

Nitrosative stress with HIV dementia causes decreased L-prostaglandin D synthase activity.

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BACKGROUND: The prevalence of HIV-associated neurocognitive disorders is increasing as HIV-infected individuals are living longer. The clinical manifestations of the syndrome also continue to evolve under the influence of antiretroviral drugs and comorbidities such as drugs of abuse. However, there are no surrogate markers for the disease, either to identify it de novo or to track its progression, and there is no proven treatment with the exception of antiretroviral drugs. METHODS: Levels of nitric oxide, nitrate, and 3-nitrotyrosine (3-NT)-modified proteins were measured in the CSF of 46 patients with HIV infection stratified according to their neurocognitive status and history of IV drug use (IVD). The 3-NT-modified proteins were isolated and identified by tandem mass spectrometry, and the functional consequence of 3-NT modification of L-prostaglandin D synthase (L-PGDS), the most abundant protein, was determined. RESULTS: 3-NT-modified proteins were significantly elevated in patients with HIV infection who had progressive neurocognitive decline over the next 6 months and in patients with a history of IVD. Thirteen different proteins with 3-NT modification

were identified in the CSF of these patients. L-PGDS was the most abundant. 3-NT modification of this protein resulted in loss of its enzymatic activity. CONCLUSIONS: There is increased nitrosative stress in CSF of HIV-infected patients with active dementia and in patients with a history of IV drug use, measurement of which may serve as a surrogate marker for these patients. Nitrosative stress may also have important functional consequences and may impact the pathogenesis of HIV-associated neurocognitive disorders.

15: Exp Gerontol 2007 Nov;

S-Glutathionylation of metallothioneins by nitrosative/oxidative stress.

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Cystein residues within metallothionein (MT) structure have been shown to be particularly prone to S-nitrosylation. The objective of this study was to examine the possibility that MTs undergo S-glutathionylation under nitrosative/oxidative stress. MT from rabbit liver was treated with different concentrations of GSNO, diamide plus GSH or H(2)O(2) plus GSH. Parallel sets of samples were treated with 10mM DTT for 30min at 37 degrees C to reduce mixed disulphides. Incubations were then processed for Western blot or dot-immunobinding assay. Western blot with anti-MT or anti-GSH were also performed on peripheral blood mononuclear cell extracts. Structural aspects of S-glutathionylation of MTs were also examined. Treatment with GSNO, diamide/GSH or H(2)O(2)/GSH induced a dose-dependent increase in the levels of MT S-glutathionylation. This effect was completely reversed by treatment with the reducing agent DTT, indicating that S-glutathionylation of MT protein was related to formation of protein-mixed disulphides. Structural analysis of rat MT indicated that Cys residues located in the N-terminal domain of the protein are the likely targets for S-glutathionylation, both for their solvent accessibility and electrostatics induced reactivity. S-Glutathionylation of MT, given its reversibility, would provide protection from irreversible oxidation of Cys residues, thus representing a mechanism of high potential biological relevance.

16: Arterioscler Thromb Vasc Biol 2008 Jan;

PARP-1 Inhibition Prevents Oxidative and Nitrosative Stress-Induced Endothelial Cell Death via Transactivation of the VEGF Receptor 2.

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OBJECTIVE: PARP-1, a DNA base repair enzyme, is activated by DNA breaks induced by oxidative (ROS) and nitrosative (RNS) stress. By consuming NAD(+), PARP-1 activation can lead to ATP depletion and cell death. Studies suggest that inhibiting PARP-1 activity can attenuate pathologies associated with vascular smooth muscle and endothelial dysfunction. PARP-1 inhibition can also activate the prosurvival serine/threonine kinase, Akt. Vascular endothelial growth factor (VEGF) regulates endothelial cell survival via Akt activation downstream of VEGF receptor 2 (VEGFR2)

activation. Here we investigated the hypothesis that PARP-1 inhibition protects human umbilical vein endothelial cells (HUVECs) from ROS- and RNS-induced cell death by limiting NAD(+) depletion and by activating a prosurvival signaling pathway via VEGFR2 phosphorylation. METHODS AND RESULTS: We activated PARP-1 in HUVECs by treatment with hydrogen peroxide (H2O2) and peroxynitrite (ONOO(-)). Both depleted HUVECs of NAD(+) and ATP, processes that were limited by the PARP-1 inhibitor, PJ34. ONOO(-) and H2O2-induced cell death and apoptosis were attenuated in cells treated with PJ34 or PARP-1 siRNA. PARP-1 inhibition increased Akt, BAD, and VEGFR2 phosphorylation in HUVECs and in PJ34-treated rabbit aortas. The VEGFR2-specific tyrosine kinase inhibitor SU1498 decreased PARP-1 inhibition-mediated phosphorylation of VEGFR2 and Akt, and also reversed survival effects of PJ34. Finally, PARP-1 inhibition protected cells from death induced by serum starvation, evidence for a role in cell survival independent of energy protection. CONCLUSIONS: PARP-1 inhibition prevents ROS- and RNS-induced HUVEC death by maintaining cellular energy in the form of NAD(+) and ATP, and also by activating a survival pathway via VEGFR2, Akt, and BAD phosphorylation.

17: Endocrinology 2007 Dec;

Stress mediators regulate brain prostaglandin synthesis and PPAR{gamma} activation after stress in rats.

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Stress exposure leads to oxidative/nitrosative and neuroinflammatory changes which have been shown to be regulated by anti-inflammatory pathways in brain. In particular, acute restraint stress is followed by COX-2 upregulation and subsequent proinflammatory prostaglandin PGE2 release in rat brain cortex. Concomitantly, the synthesis of the anti-inflammatory prostaglandin 15d-PGJ2 and the activation of its nuclear target the peroxisome proliferator-activated receptor gamma (PPARgamma) are also produced. This study is aimed to determine the possible role of the main stress mediators: catecholamines, glucocorticoids and excitatory amino acids (glutamate) in the above mentioned stress-related effects. By using specific pharmacological tools, our results show that the main mediators of the stress response are implicated in the regulation of prostaglandin synthesis and PPARgamma activation in rat brain cortex described after acute restraint stress exposure. Pharmacological inhibition (predominantly through beta-adrenergic receptor) of the stress-released catecholamines in CNS regulates 15d-PGJ2 and PGE2 synthesis, by reducing COX-2 overexpression, and reduces PPARgamma activation. Stress-produced glucocorticoids (GCs) carry out their effects on prostaglandin synthesis through their interaction with mineralocorticoid (MR) and glucocorticoid receptors (GR) in a very similar degree. However, in the case of PPARgamma regulation only the actions through GR seem to be relevant. Finally, the selective blockade of the NMDA type of glutamate receptor after stress also negatively regulates 15d-PGJ2 and PGE2 production by COX-2 downregulation and decrease in PPARgamma transcriptional activity and expression. In conclusion, we show here that the main stress mediators: catecholamines, GCs and glutamate, concomitantly regulate the activation of proinflammatory and anti-inflammatory pathways in a possible co-regulatory mechanism of the inflammatory process induced in rat brain cortex by acute restraint stress exposure.

20: Arch Toxicol 2008 Feb;

Methotrexate-induced nitrosative stress may play a critical role in small intestinal damage in the rat.

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Methotrexate (MTX), a structural analogue of folic acid, is widely used as a chemotherapeutic agent for leukemia and other malignancies. One of the major toxic effects of MTX is intestinal injury and enterocolitis .The mechanism of gastrointestinal toxicity of methotrexate has not been investigated completely. Therefore cancer chemotherapy has to be accompanied by symptomatic therapy such as antibiotics and anti-diarrheal drugs. It is important to investigate the mechanism by which methotrexate induces intestinal damage in order to perform cancer chemotherapy effectively by preventing the side effects. This study aimed at investigating whether nitrosative stress plays a role in methotrexate induced small intestinal damage using a rat model. Adult male rats were administered methotrexate at the dose of 7 mg/kg body weight intraperitoneally for 3 consecutive days and sacrificed 12 or 24 h after the final dose of methotrexate. Vehicle treated rats served as control. The intestinal tissue was used for light microscopic studies and markers of nitrosative stress including tissue nitrite level and nitrotyrosine. Myeloperoxidase (MPO) activity, a marker of neutrophil infiltration was also measured in intestinal homogenates. The villi were damaged at 12 h and the damage progressed and became severe at 24 h after the final dose of MTX. Biochemically, tissue nitrate was elevated fivefold at 12 h and fourfold at 24 h after the final dose of MTX as compared with control. Nitrotyrosine, measured immunohistochemically was detected in all the parts of the small intestine. Duodenum stained the most for nitrotyrosine, followed by ileum and then jejunum. The staining for nitrotyrosine was more intense at 24 h as compared with 12 h after the final dose of methotrexate. There was marked neutrophil infiltration as evidenced by increase in MPO activity in the small intestines. In conclusion, the results of the present study reveal that nitrosative stress may play a critical role in methotrexate induced small intestinal damage. Intervention studies using nitric oxide synthase inhibitors is being carried out in order to confirm the role of nitrosative stress in methotrexate induced small intestinal damage.

23: J Am Coll Cardiol 2008 Aug;52(8):655-66

Metallothionein suppresses angiotensin II-induced nicotinamide adenine dinucleotide phosphate oxidase activation, nitrosative stress, apoptosis, and pathological remodeling in the diabetic heart.

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OBJECTIVES: We evaluated metallothionein (MT)-mediated cardioprotection from angiotensin II (Ang II)-induced pathologic remodeling with and without underlying diabetes. BACKGROUND: Cardiac-specific metallothionein-overexpressing transgenic (MT-TG) mice are resistant to diabetic

cardiomyopathy largely because of the antiapoptotic and antioxidant effects of MT. METHODS: The acute and chronic cardiac effects of Ang II were examined in MT-TG and wild-type (WT) mice, and the signaling pathways of Ang II-induced cardiac cell death were examined in neonatal mouse cardiomyocytes. RESULTS: Acute Ang II administration to WT mice or neonatal cardiomyocytes increased cardiac apoptosis, nitrosative damage, and membrane translocation of the nicotinamide adenine dinucleotide phosphate oxidase (NOX) isoform p47(phox). These effects were abrogated in MT-TG mice, MT-TG cardiomyocytes, and WT cardiomyocytes pre-incubated with peroxynitrite or superoxide scavengers and NOX inhibitors, suggesting a critical role for NOX activation in Ang II-mediated apoptosis. Prolonged administration of subpressor doses of Ang II (0.5 mg/kg every other day for 2 weeks) also induced apoptosis and nitrosative damage in both diabetic and nondiabetic WT hearts, but not in diabetic and nondiabetic MT-TG hearts. Long-term follow-up (1 to 6 months) of both WT and MT-TG mice after discontinuing Ang II administration revealed progressive myocardial fibrosis, hypertrophy, and dysfunction in WT mice but not in MT-TG mice. CONCLUSIONS: Metallothionein suppresses Ang II-induced NOX-dependent nitrosative damage and cell death in both nondiabetic and diabetic hearts early in the time course of injury and prevents the late development of Ang II-induced cardiomyopathy.

24: Neuroscience 2008 Aug;

Enhanced stress reactivity in nitric oxide synthase 2 mutant mice: Findings in support of astrocytic nitrosative modulation of behavior.

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Alterations of nitric oxide (NO) metabolism in the brain have been associated with modifications of stress-related behavior in animal models. It has been generally assumed that these behavioral changes are due to the neuronal nitrosative activity. On the other hand, glial NO production has been demonstrated mainly as a slow reaction to brain insults through the activity of an inducible nitric oxide synthase (NOS) isoform (NOS2). Recently we uncovered increased NOS activity in astrocytes of mice with a NOS2 mutation. Interestingly, these mice revealed a behavioral phenotype suggestive of increased susceptibility to stress. In the present study we investigated the responses of these mutants to stress by exposing them to predator scent. Seven days later, mutant mice exhibited significantly higher anxiety-like behavior in the elevated-plus maze, increased acoustic startle responses, and higher plasma corticosterone levels compared with their controls. Systemic administration of a NOS inhibitor prior to the stress exposure reversed these stress-related effects without affecting controls' behavior. These findings are in agreement with previous studies showing an association between increased NO levels and enhanced anxiety-like responses. In addition, mutant mice performed better in the Morris water maze prior to stress exposure, but the two animal groups performed alike in an object-recognition test. Taken together, our results suggest the involvement of astrocytic-derived NO in modulating behavior.

25: Neuro Endocrinol Lett 2008 Jun;29(3)

The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Minireview.

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This paper hypothesizes that inflammatory, oxidative and nitrosative (IO&NS) pathways, and an increased translocation of LPS from gram-negative bacteria are causally related to depression following external (psychological) and internal (organic) stressors and that IO&NS pathways are novel targets for antidepressant development. We review that depression is accompanied by an inflammatory reaction as indicated by an increased production of pro-inflammatory cytokines, such as interleukin-1beta (IL-1beta), IL-6, tumour necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN)-gamma. These cytokines are stress-sensitive and may cause depressive behaviors. The latter may be induced by an increased catabolism of tryptophan, the precursor of serotonin, to neurotoxic TRYCATs (tryptophan catabolites along the indoleamine oxidase pathway). Inflammatory biomarkers are detected in animal models of depression. Newly developed animal models of depression are based on induced inflammation. Most if not all antidepressants have specific anti-inflammatory effects. Anti-inflammatory compounds may augment the clinical efficacy of antidepressants. Depression is also accompanied by an IgM-related (auto)immune response directed against disrupted lipid membrane components, such as phosphatidyl-inositol, by-products of lipid peroxidation, e.g. azelaic acid and malondialdehyde, and NO-modified amino-acids, which are normally not detected by the immune system but due to damage caused by O&NS have become immunogenic. Increased translocation of lipopolysaccharide from gram-negative bacteria, which may be induced by internal and external stressors, may further aggravate the induced IO&NS pathways. Future research to disentangle the complex pathophysiology of depression calls for a powerful paradigm shift, i.e. using a high throughput screening according to the translational medicine methodology.

30: Comp Biochem Physiol B Biochem Mol Biol 2008 Jun;

Hyperthermic stress-induced increase in the expression of glutamate-cysteine ligase and glutathione levels in the symbiotic sea anemone Aiptasia pallida.

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Hyperthermic stress is known to trigger the loss of unicellular algae from a number of symbiotic cnidarians, a phenomenon commonly referred to as bleaching. Oxidative and nitrosative stress have been suggested to play a major role during the process of bleaching, however the underlying molecular mechanisms are still poorly understood. In animals, the intracellular tripeptide glutathione (GSH) is involved in antioxidant defense, redox homeostasis and intracellular redox signaling. Therefore, we tested the hypothesis that hyperthermal stress-induced bleaching in Aiptasia pallida, a model for symbiotic cnidarians, results in increased levels of GSH synthesis. We report the cDNA sequence and functional analysis of the catalytic subunit of glutamate-cysteine ligase (GCLC), which catalyzes the rate-limiting step in GSH biosynthesis. In a time-series experiment, both GCLC gene expression and total GSH levels increased

4- and 1.5-fold, respectively, in response to hyperthermal stress. These results suggest that hyperthermal stress triggers adaptive increases in intracellular GSH biosynthesis in cnidarians as a protective response to oxidative/nitrosative stress. Our results show the conserved function of GCLC and GSH across animals while placing a new perspective on the role of GSH in redox signaling during cnidarian bleaching.

31: Inflamm Allergy Drug Targets 2008 Sep;7(3):129-35

Potassium Channel Openers and Improvement of Toxic Stress: Do they have Role in the Management of Inflammatory Bowel Disease?

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Inflammatory bowel disease (IBD) is a progressive condition in gastrointestinal tract, which refers to two idiopathic diseases; ulcerative colitis and Crohn's disease. Although certain etiology of these conditions is not known, it seems that an abnormality in reaction and regulation of the immune system plays an important role in adventure of the disease. According to the investigations, it is likely that oxidative and nitrosative stress have etiologic roles in IBD. Their destructive effects may contribute to the initiation or progression of the disease. Nowadays, the effectiveness of different medicines in the treatment of IBD has been proved, but none of them has shown a desirable result. Potassium channel openers (PCOs) are a class of drugs with various usages in the aspects of cardiovascular diseases and urinary incontinence. Their major mechanism is the opening of ATP-sensitive potassium (K-ATP) channels and contribute to the relaxation of smooth muscles. Nicorandil is a member of PCOs, with a special chemical structure. Recent investigations mention some novel effects and functions for this drug. Nicorandil reveals an anti-apoptosis property not only via a nitric oxide (NO)/cGMP-dependent mechanism, but also through activating mitochondrial K-ATP channels. Nicorandil can also elevate cGMP levels in some tissues, without direct NO generation. Gastroprotective activity via opening of the K channels, free radical scavenging, prostaglandin E2 elevation, decreasing pepsin and acid secretion, and prevention of the detrimental rise in NO has been proposed for nicorandil. According to these protective mechanisms and the role of oxidative/nitrosative stress in the expression of IBD, we herein hypothesize that nicorandil and other PCOs with similar structure can be used in the management of IBD. This approach offers new hope for the successful treatment of IBD. Further investigations on animal models are needed, to place nicorandil and similar drugs alongside IBD therapy.

32: J Med Food 2008 Sep;11(3):582-6

Garlic powder ameliorates Cisplatin-induced nephrotoxicity and oxidative stress.

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ABSTRACT cis-Diamminedichloroplatinum (II) (cisplatin) is an effective chemotherapeutic agent successfully used in the treatment of a wide range of tumors. Nevertheless, nephrotoxicity has restricted its clinical use. The use of more than a few antioxidants has shown that reactive oxygen species are involved in cisplatin-induced nephrotoxicity. In the present work the effect of garlic powder, a recognized antioxidant, on cisplatin-induced nephrotoxicity and oxidative and nitrosative stress was studied. Rats were fed with a 2% garlic powder diet for 4 weeks. A single injection of cisplatin (7.5 mg/kg) induced tubular damage and an increase in the following markers of renal injury 3 days later: blood urea nitrogen, serum creatinine, and urinary excretion of N-acetyl-beta-D-glucosaminidase. The cisplatin injection also increased 3-nitrotyrosine and 4-hydroxy-2-nonenal immunostaining in renal cortex and medulla. It was found that the garlic powder feeding was able to prevent by 40-59% the alterations in the markers of renal injury studied, by 33% the histological damage, and by 38-75% the increase in markers of oxidative and nitrosative stress. It is concluded that the ability of garlic powder to ameliorate cisplatin-induced renal injury is associated with its antioxidant properties. Our data support the use of garlic powder as a renoprotective agent.

33: Antioxid Redox Signal 2008 Aug;

Targeting Oxidative Stress for Neuroprotection.

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The generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to oxidative and/or nitrosative damage to cellular proteins, lipids and DNA (a process collectively referred to here as oxidative stress). During ageing, oxidative stress increases due to an aberrant generation of ROS/RNS and a gradual decline in cellular antioxidant defense mechanisms. Consequently, ageing and the associated increase in oxidative stress are major risk factors for many neurodegenerative diseases. In addition, various genetic mutations and environmental exposures can sensitize individuals to oxidative stress and neurodegeneration. Within the cell, mitochondria are a major source of oxidative stress. However, additional intracellular sources of ROS and RNS exist, as well as extracellular sources such as those resulting from inflammation or exposure to toxins. A significant body of literature indicates that ROS and/or RNS resulting from mitochondrial dysfunction, neuroinflammation, or toxicants are major factors in the oxidative stress-dependent neuronal death that underlies various neurodegenerative disorders including Parkinsons disease (PD), Alzheimers disease (AD), Huntingtons disease (HD), amyotrophic lateral sclerosis (ALS), and many others (1, 8, 9, 16, 18). Accordingly, the discovery of novel strategies to mitigate oxidative stress is a principal focus of current therapeutic development programs for neurodegenerative diseases.

34: J Cell Biochem 2008 May;

PARP-1 modulates deferoxamine-induced HIF-1alpha accumulation through the regulation of nitric oxide and oxidative stress.

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Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear protein that, once activated by genotoxic agents, modulates the activity of several nuclear proteins including itself. Previous studies have established that PARP-1 inhibition may provide benefit in the treatment of different diseases, particularly those involving a hypoxic situation, in which an increased oxidative and nitrosative stress occurs. One of the most important transcription factors involved in the response to the hypoxic situation is the hypoxia-inducible factor-1 (HIF-1). The activity of HIF-1 is determined by the accumulation of its alpha subunit which is regulated, in part, by oxidative stress (ROS) and nitric oxide (NO), both of them highly dependent on PARP-1. Besides, HIF-1alpha can be induced by iron chelators such as deferoxamine (DFO). In this sense, the therapeutical use of DFO to strengthen the post-hypoxic response has recently been proposed. Taking into account the increasing interest and potential clinical applications of PARP inhibition and DFO treatment, we have evaluated the impact of PARP-1 on HIF-1alpha accumulation induced by treatment with DFO. Our results show that, in DFO treated cells, PARP-1 gene deletion or inhibition decreases HIF-1alpha accumulation. This lower HIF-1alpha stabilization is parallel to a decreased inducible NO synthase induction and NO production, a higher response of some antioxidant enzymes (particularly glutathione peroxidase and glutathione reductase) and a lower ROS level. Taken together, these results suggest that the absence of PARP-1 modulates HIF-1 accumulation by reducing both NO and oxidative stress. J. Cell. Biochem. (c) 2008 Wiley-Liss, Inc.

35: Int J Radiat Biol 2008 Aug;84(8):669-80

The protective effects of N-acetyl-L-cysteine and Epigallocatechin-3-gallate on electric field-induced hepatic oxidative stress.

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Purpose: To investigate the effects of 12 kV/m electric (E) field sourced by power lines on oxidative and nitrosative stress, and antioxidant status. Furthermore, the study aimed to examine the protective effects of N-Acetyl-L-cysteine (NAC) and epigallocatechin-gallate (EGCG) in the liver tissues of guinea pigs against the possible detriments of electromagnetic field exposure. Materials and methods: Guinea pigs were exposed to 50 Hz 12 kV/m E-field. NAC and EGCG were administerated intraperitoneally. Malonedialdehyde (MDA), a product of lipid peroxidation (LPO), and nitric oxide derivatives (nitrate (NO(3)), nitrite (NO(2)), total level of nitric oxide (NO(x)) were estimated as biomarkers of oxidative and nitrosative stress, respectively. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and myeloperoxidase (MPO) were evaluated as endogenous antioxidant enzymes in liver tissues of the guinea pigs. Results: The results of our study indicated a significant increase in the levels of oxidant products (MDA, NO(3), NO(2), NO(x)), and a significant decrease in antioxidant enzyme (SOD, GSH-Px and MPO) activities. We also found that the individual or plus application of NAC and EGCG resulted in the reduction of oxidative stress prior to E field application. Conclusion: To conclude, extremely low frequency (ELF) electric field has potential harmful effects

on the living organisms by enhancing the free radical production. NAC and EGCG might have hepatoprotective effects in ELF-E field induced oxidative and nitrosative stress.

36: J Bacteriol 2008 May;

The Campylobacter jejuni thiol peroxidases Tpx and Bcp both contribute to aerotolerance and peroxide-mediated stress resistance but have distinct substrate specificities.

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The microaerophilic food-borne pathogen Campylobacter jejuni experiences variable oxygen concentrations during its life-cycle, especially during transitions between the external environment and the avian or mammalian gut. Single knockout mutations in either one of two related thiol peroxidase genes, tpx and bcp, resulted in normal microaerobic growth (10% v/v oxygen) but poorer growth than the wild-type under high aeration conditions (21 % v/v oxygen). However, a tpx/bcp double mutant had a severe microaerobic growth defect and did not grow at high aeration in shake flasks. Although the single mutant strains were no more sensitive than the wild-wild-type in disc diffusion assays with hydrogen peroxide, organic peroxides, superoxide or nitrosative stress agents, in all cases the double mutant was hypersensitive. Quantitative cell viability and cellular lipid peroxidation assays indicated some increased sensitivity of the single tpx and bcp mutants to peroxide stress. Protein carbonylation studies revealed that the tpx/bcp double mutant had a higher degree of oxygen- and peroxide-induced oxidative protein damage than either of the single mutants. An analysis of the peroxidase activity of the purified recombinant enzymes showed that, surprisingly, Tpx only reduced hydrogen peroxide as substrate, whereas Bcp also reduced organic peroxides. Immunoblotting of wild-type cell-free extracts with Tpx or Bcp specific antibodies showed increased abundance of both proteins under high aeration compared to microaerobic growth conditions. Taken together, the results suggest that Tpx and Bcp are partially redundant antioxidant enzymes that play an important role in protection of C. jejuni against oxygen-induced oxidative stress.

37: Cell Biochem Funct 2008 Mar;

The effects of rosiglitazone on oxidative stress and lipid profile in left ventricular muscles of diabetic rats.

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We investigated the effect of rosiglitazone (RSG), a high-affinity ligand for the peroxisome proliferator-activated receptor gamma which mediates insulin-sensitizing actions, on the lipid profile and oxidative status in streptozotocin (STZ)-induced Type 2 diabetes mellitus (DM) rats. Wistar albino male rats were randomly divided into an untreated control group (C),

a C + RSG group which was treated with RSG (4 mg kg(-1)) two times a day by gavage, a diabetic group (D) that was treated with a single intraperitoneal injection of STZ (45 mgkg(-1)), D + RSG group which were treated with RSG two times a day by gavage, respectively. Lipid profiles, HbA(1c) and blood glucose levels in the circulation and malondialdehyde (MDA) and 3-nitrotyrosine (3-NT) levels in left ventricular muscle were measured. Treatment of D rats with RSG resulted in a time-dependent decrease in blood glucose. We found that the lipid profile and HbA(1c) levels in D + RSG group reached the C rat values at the end of the treatment period. There was a statistically significant difference between the C + RSG and C groups in 3-NT levels. In group D, 3-NT and MDA levels were found to be increased when compared with C, C + RSG and D + RSG groups. In the D + RSG group, MDA levels were found to be decreased when compared with C and C + RSG. Our study suggests that the treatment of D rats with RSG for 8 weeks may decrease the oxidative/nitrosative stress in left ventricular tissue of rats. Thus in diabetes-related vascular diseases, RSG treatment may be cardioprotective.

38: Hum Exp Toxicol 2008 Jun;27(6):463-469

Study on the oxidative stress status among cement plant workers.

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The cement industry is considered as a major pollution problem because of dust and particulate matter emitted at various steps of cement production. In the present study, volunteer male workers from a cement factory were studied for oxidative and nitrosative stress biomarkers in relation to their serum levels of aluminum (Al) and chromium (Cr). The subjects were divided into two groups of direct and indirect exposure. Subject who worked in production steps were considered as direct exposure group, and those who worked in administration building were considered as indirect exposure group. For comparison, healthy subjects at the same age and socioeconomic status were tested as a control group. Serum levels of lipid peroxidation (LP), total antioxidant capacity (TAC), total thiol molecules (TTM), and nitric oxide (NO) as well as Al and Cr were measured. The results indicated a significant increase in Al ($P = 0.001$) and Cr ($P = 0.009$) levels in direct-exposed workers in comparison to healthy control group. Further, a significant increase in Al ($P = 0.002$) and Cr ($P = 0.009$) levels was observed in direct-exposed workers as compared to indirect-exposed one. Serum levels of TTM and TAC were significantly lower in both direct- and indirect-exposed groups in comparison to healthy control group ($P = 0.00$). Serum TTM and TAC were significantly lower in direct-exposed workers as compared to indirect-exposed ones ($P = 0.00$ and $P = 0.024$, respectively). There was no significant difference on the level of LP and NO among groups. A correlation was found between serum level of Cr, TAC, and platelets between direct- and indirect-exposed groups ($P < 0.05$). Further correlation was found among serum level of Cr and those of TTM, platelets, and chronic disease ($P < 0.05$). Chronic disease had a significant influence adjusted to other predictor variables on the post-shift values of Al ($P < 0.05$). Although plasma levels of Al and Cr were found in normal ranges, analyses confirm their role in impairment of TMM and TAC.

39: Chin J Physiol 2008 Apr;51(2):85-93

Oxidative and nitrosative mediators in hepatic injury caused by whole body hyperthermia in rats.

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The involvement of oxidative and nitrosative mediators in liver injury caused by heat stress remains unclear. This study aimed to elucidate the role of endothelial nitric oxide synthase (eNOS), and inducible NOS (iNOS)-derived NO and nitrotyrosine in the whole-body hyperthermia (WBH)-induced liver injury. Rats were anesthetized with intraperitoneal pentobarbital, and were exposed to a heating lamp for 60 min to raise the core temperature to 42.5 degrees C. The rats were maintained at the hyperthermic state for an additional 50 min. Blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, lactic dehydrogenase, creatine phosphokinase, amylase, lipase, nitrate/nitrite, methyl guanidine, and proinflammatory cytokines (tumor necrosis factoralpha, interleukin-1beta and interleukin-10) were measured before and 14 h after hyperthermia. Immunohistochemical staining was employed to detect the eNOS, iNOS and nitrotyrosine levels. Western blotting was used to examine the expression of heatshock protein 70 (HSP 70). Histopathological examination of the liver tissue was performed. WBH caused liver injury accompanied with significant increases in biochemical factors, nitrate/nitrite, methyl guanidine, and proinflammatory cytokines. In addition, WBH enhanced the eNOS, iNOS, nitrotyrosine and HSP 70 levels. WBH caused hepatic injury. The pathogenetic mechanism is likely mediated through the NOS-derived NO, free radical, proinflammatory cytokines and nitrotyrosine. The enhanced expression of HSP 70 may play a protective role.

40: Stroke 2008 Feb;

Toll-Like Receptor 4 Is Involved in Subacute Stress-Induced Neuroinflammation and in the Worsening of Experimental Stroke.

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BACKGROUND AND PURPOSE: Psychological stress causes an inflammatory response in the brain and is able to exacerbate brain damage caused by experimental stroke. We previously reported that subacute immobilization stress in mice worsens stroke outcome through mechanisms that involve inflammatory mechanisms, such as accumulation of oxidative/nitrosative mediators and expression of inducible nitric oxide synthase and cyclooxygenase-2 in the brain. Some of these inflammatory mediators could be regulated by innate immunity, the activation of which takes place in the brain and produces an inflammatory response mediated by toll-like receptors (TLRs). Recently, we described the implications of TLR4 in ischemic injury, but the role of TLR4 in stress has not yet been examined. We therefore investigated whether inflammation produced by immobilization stress differs in mice that lack a functional TLR4 signaling pathway. METHODS: We used an experimental paradigm consisting of the exposure of mice to repeated immobilization sessions (1 hour daily for 7 days) before permanent middle cerebral artery occlusion. RESULTS: We found that TLR4-deficient mice

subjected to subacute stress had a better behavioral condition compared with normal mice (C3H/HeN) and that this effect was associated with a minor inflammatory response (cyclooxygenase-2 and inducible nitric oxide synthase expression) and lipid peroxidation (malondialdehyde levels) in brain tissue. Furthermore, previous exposure to stress was followed by a smaller infarct volume after permanent middle cerebral artery occlusion in TLR4-deficient mice than in mice that express TLR4 normally. CONCLUSIONS: Our results indicate that TLR4 is involved in the inflammatory response after subacute stress and its exacerbating effect on stroke. These data implicate the effects of innate immunity on inflammation and damage in the brain after stroke.

41: Toxicology 2007 Dec;

Protective effects of apocynin against cisplatin-induced oxidative stress and nephrotoxicity.

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Cis-diamminedichloroplatinum (II) (cisplatin) is an effective chemotherapeutic agent successfully used in the treatment of a wide range of tumors; however, nephrotoxicity has restricted its clinical use. Several studies have shown that reactive oxygen species are involved in cisplatin-induced nephrotoxicity, including hydrogen peroxide, hydroxyl radical and superoxide anion (O(2)(-)). The source of O(2)(-) in cisplatin-induced renal damage has not been established. The aim of this study was to investigate if NADPH oxidase is involved in cisplatin-induced nephrotoxicity using apocynin, a widely used NADPH oxidase inhibitor. Rats were studied 3 days after a single injection of cisplatin (7.5mg/kg, i.p.). Apocynin was given in the drinking water (2g/L) 7 days before and 3 days after cisplatin injection. Apocynin treatment was able to ameliorate the renal histological damage and the increase in blood urea nitrogen, serum creatinine, and urinary excretion of total protein, N-acetyl-beta-d-glucosaminidase and glutathione-S-transferase induced by cisplatin. In addition, the protective effect of apocynin was associated with the amelioration of cisplatin-induced oxidative and nitrosative stress. Our data suggest that O(2)(-) derived from NADPH oxidase triggers some of the side effects due to cisplatin administration.

42: Food Chem Toxicol 2007 Nov;

Nordihydroguaiaretic acid attenuates potassium dichromate-induced oxidative stress and nephrotoxicity.

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Larrea tridentata also known as Creosote bush, Larrea, chaparral, greasewood or gobernadora has been used in the folk medicine for the treatment of several illnesses. The primary product that is present at high concentrations in the leaves from this plant is nordihydroguaiaretic acid (NDGA) which is a powerful antioxidant. On the other hand, potassium dichromate (K(2)Cr(2)O(7))-induced nephrotoxicity is associated with oxidative stress. The aim of this work was to study the effect of NDGA on K(2)Cr(2)O(7)-induced nephrotoxicity and oxidative stress. Nephrotoxicity was induced by a single injection of K(2)Cr(2)O(7) (15mg/Kg). A group of K(2)Cr(2)O(7)-treated rats was administered NDGA by mini osmotic pumps (17mg/Kg/day). The results show that NDGA was able to ameliorate the structural and functional renal damage evaluated by histopathological analysis and by measuring proteinuria, urinary excretion of N-acetyl-beta-d-glucosaminidase, serum creatinine, and serum glutathione peroxidase activity. In addition, immunostaining of 4-hydroxy-2-nonenal and 3-nitrotyrosine, markers of oxidative and nitrosative stress, respectively, was ameliorated by the NDGA treatment. These data strongly suggest that the antioxidant properties of NDGA are involved in its renoprotective effect in K(2)Cr(2)O(7)-treated rats.

43: J Asthma 2008 Mar;45(2):149-54

The value of sputum 8-isoprostane in detecting oxidative stress in mild asthma.

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Background. Exhaled nitric oxide and induced sputum eosinophils are well established as direct markers of inflammation/oxidative stress in asthma. Recently, it has been proposed that sputum 8-isoprostane concentrations may present a reliable index for measuring oxidative stress in asthmatic patients. We assessed the value of sputum 8-isoprostane in mild asthma in children and adolescents. Methods. Patients with newly diagnosed asthma (children, n = 23; adults, n = 14) and age-matched healthy controls (children, n = 13; adults, n = 15) were studied. Lung function was measured by spirometry, sputum was induced by hypertonic saline, and fractional exhaled nitric oxide (FeNO) was measured with standard methods. Cell differential counts were obtained from sputum slides and the concentration of 8-isoprostane was measured with an enzyme immunoassay from sputum supernatants. Results. High-quality sputum specimens could be obtained from 10 children and 10 adults, and the sputum analyses were conducted only for the representative specimens. Asthmatics had increased FeNO (children 35.5 vs. 11.9 ppb; adults 81.1 vs. 16.6 ppb; $p < 0.001$) and sputum eosinophils (children 2.4% vs. 1.4%; adults 10.4% vs. 0.2%; $p = 0.005$) compared to healthy controls. There was a significant correlation between FeNO and eosinophils ($R = 0.65$; $p < 0.0001$). Sputum 8-isoprostane was not elevated in asthmatics compared to healthy subjects (children 81.1 vs. 89.9 and adults 76.9 vs. 73.4 pg/mL) and did not correlate with lung function or other measurements of airway inflammation. However, increased 8-isoprostane levels were detected in patients with chronic obstructive pulmonary disease (n = 11, 184.7 pg/mL, used as controls for assays). Conclusions. In agreement with earlier studies, FeNo is sensitive in detecting oxidative/nitrosative stress in asthmatic airways. However, our results suggest that 8-isoprostane may not be sensitive in reflecting oxidant burden in mild asthma.

44: J Bacteriol 2008 Jan;

Widespread distribution in pathogenic bacteria of di-iron proteins that repair oxidative and nitrosative damage to iron-sulfur centers.

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Expression of two genes of unknown function, Staphylococcus aureus scdA and Neisseria gonorrhoeae dnrN, is induced by exposure to oxidative or nitrosative stress. We show that DnrN and ScdA are di-iron proteins that protect their hosts from damage caused by exposure to nitric oxide and to hydrogen peroxide. Loss of FNR-dependent activation of aniA expression and NsrR-dependent repression of norB and dnrN expression on exposure to NO was restored in the gonococcal parent strain, but not in a dnrN mutant, suggesting that DnrN is necessary for the repair of NO damage to the gonococcal transcription factors, FNR and NsrR. Restoration of aconitase activity destroyed by exposure of S. aureus to NO or H2O2 required a functional scdA gene. EPR spectra of recombinant ScdA purified from E. coli confirmed the presence of a di-iron center. The recombinant scdA plasmid, but not recombinant plasmids encoding the complete E. coli sufABCDSE or iscRSUAhscBAfdx operons, complemented repair defects of an E. coli ytfE mutant. Analysis of the protein sequence database revealed the importance of the two proteins based on the widespread distribution of highly conserved homologues in both Gram-positive and Gram-negative bacteria that are human pathogens. We provide in vivo and in vitro evidence that Fe-S clusters damaged by exposure to NO and H2O2 can be repaired by this new protein family, for which we propose the name Repair of Iron Centers proteins.

45: Biochem Soc Trans 2008 Oct;36(Pt 5):1045-50

Dicarbonyls linked to damage in the powerhouse: glycation of mitochondrial proteins and oxidative stress.

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Protection of mitochondrial proteins from glycation by endogenous dicarbonyl compounds, methylglyoxal and glyoxal, was found recently to prevent increased formation of reactive oxygen species and oxidative and nitrosative damage to the proteome during aging and produce life extension in the nematode Caenorhabditis elegans. This suggests that dicarbonyl glycation damage to the mitochondrial proteome may be a preceding event to mitochondrial dysfunction leading to oxidative stress. Future research will address the functional charges in mitochondrial proteins that are the targets for dicarbonyl glycation.

46: Proc Natl Acad Sci U S A 2008 Aug;

Regulation of the SigH stress response regulon by an essential protein kinase in Mycobacterium tuberculosis.

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SigH is a key regulator of an extensive transcriptional network that responds to oxidative, nitrosative, and heat stresses in Mycobacterium tuberculosis, and this sigma factor is required for virulence in animal models of infection. SigH is negatively regulated by RshA, its cognate anti-sigma factor, which functions as a stress sensor and redox switch. While RshA provides a direct mechanism for sensing stress and activating transcription, bacteria use several types of signal transduction systems to sense the external environment. M. tuberculosis encodes several serine-threonine protein kinase signaling molecules, 2 of which, PknA and PknB, are essential and have been shown to regulate cell morphology and cell wall synthesis. In this work, we demonstrate that SigH and RshA are phosphorylated in vitro and in vivo by PknB. We show that phosphorylation of RshA, but not SigH, interferes with the interaction of these 2 proteins in vitro. Consistent with this finding, negative regulation of SigH activity by RshA in vivo is partially relieved in strains in which pknB is over-expressed, resulting in increased resistance to oxidative stress. These findings demonstrate an interaction between the signaling pathways mediated by PknB and the stress response regulon controlled by SigH. The intersection of these apparently discrete regulatory systems provides a mechanism by which limited activation of the SigH-dependent stress response in M. tuberculosis can be achieved. Coordination of the PknB and SigH regulatory pathways through phosphorylation of RshA may lead to adaptive responses that are important in the pathogenesis of M. tuberculosis infection.

47: BMB Rep 2008 Mar;41(3):194-203

Nitrosative protein tyrosine modifications: biochemistry and functional significance.

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Nitrosative modifications regulate cellular signal transduction and pathogenesis of inflammatory responses and neurodegenerative diseases. Protein tyrosine nitration is a biomarker of oxidative stress and also influences protein structure and function. Recent advances in mass spectrometry have made it possible to identify modified proteins and specific modified amino acid residues. For analysis of nitrated peptides with low yields or only a subset of peptides, affinity 'tags' can be bait for 'fishing out' target analytes from complex mixtures. These tagged peptides are then extracted to a solid phase, followed by mass analysis. In this review, we focus on protein tyrosine modifications caused by nitrosative stresses and proteomic methods for selective enrichment and identification of nitrosative protein modifications.

48: Kidney Int 2008 Oct;74(7):969

Pitfalls in the measurement of tissue DDAH activity: is DDAH sensitive to nitrosative and oxidative stress?

50: Exp Clin Endocrinol Diabetes 2008 Aug;

Nitrosylated Proteins in Monocytes as a new Marker of Oxidative-Nitrosative Stress in Diabetic Subjects with Macroangiopathy.

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BACKGROUND: Peroxynitrite plays an important role in the pathogenesis of diabetic complications. Nitrosylated protein expression in peripheral blood monocytes reflects intracellular peroxynitrite injury, and thus could be a marker of higher diagnostic and prognostic value than plasma nitrotyrosine level. The purpose of this pilot study was to assess if peripheral blood monocytes of diabetic subjects accumulate nitrosylated proteins, and if nitrosylated protein expression correlates with blood glucose control, variables of lipid profile, C-reactive protein concentration (a marker of inflammation), and differs in patients with and without diabetic macrovascular and microvascular complications. METHODS: Nitrosylated protein expression in peripheral blood monocytes (Western blot analysis) was assessed in 31 subjects with diabetes mellitus (29 Type 2, 2 Type 1; 20 males, 11 females; mean age 66 years). The presence of microangiopathy was defined by retinopathy, albumin excretion, and/or neuropathy, and macroangiopathy by carotid plaques, a history of myocardial infarction, and/or stroke. RESULTS: Diabetic subjects accumulated significant amounts of nitrosylated proteins in peripheral blood monocytes. Nitrosylated protein expression positively correlated with body weight, blood glucose, HbA (1)C, and plasma C-reactive protein concentrations in the whole cohort as well as in subjects with diabetic macroangiopathy. CONCLUSIONS: Monocyte nitrosylated protein expression is a new biomarker of metabolic control and inflammation in diabetic subjects with macroangiopathy. A more detailed assessment of diabetic microvascular complications in a larger group of patients is needed to determine if this variable can be employed as a biomarker of the presence, severity, and progression of diabetic neuropathy, retinopathy, and nephropathy.

51: Eur J Clin Invest 2008 Jul;38(7):523-30

Severe liver steatosis correlates with nitrosative and oxidative stress in rats.

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Background Little is known about nitric oxide (NO) metabolism and redox changes with hepatocyte adipocytic transformation. The aims of this study were to investigate the changes occurring in plasma and hepatic NO metabolites and redox balance in a rat experimental model of simple fatty liver, and to relate plasma with hepatic and mitochondrial changes at

different degrees of steatosis. Materials and methods Circulating and hepatic redox active and nitrogen regulating molecules thioredoxin, glutathione, protein thiols (PSH), mixed disulfides (PSSG), NO metabolites nitrosothiols, nitrite plus nitrate (NOx), and lipid peroxides (TBARs) were measured in rats fed a choline deprived (CD) diet for 30 days. Results At histology, the CD diet resulted in hepatocellular steatosis (75% of liver weight at day 30) with no signs of necro-inflammation. In plasma, thioredoxin, nitrosothiols and NOx were unchanged, while TBARs levels increased significantly and were positively related with hepatic TBARs ($r = 0.87$, $P < 0.001$) and lipid content ($r = 0.90$, $P < 0.001$). In the liver, glutathione initially increased (day 3) and then decreased. From day 14, PSH decreased and NO derivatives increased. Thioredoxin 1 had initially increased (days 7–14) and then decreased. In the mitochondria, on day 14, nitrosothiols were inversely related to thioredoxin 2 ($r = 0.988$, $P < 0.05$); on day 30, PSH were decreased by 70%, PSSG were doubled and related with nitrosothiols levels ($r = 0.925$, $P < 0.001$). Conclusion Adipocytic transformation of hepatocytes is accompanied by major interrelated modifications of redox parameters and NO metabolism especially at mitochondrial level, suggesting an early adaptive protective response but also an increased predisposition towards pro-oxidant insults.

52: Gut 2007 Oct;

Disruption of gastric barrier function by luminal nitrosative stress: A potential chemical insult to human gastro-oesophageal junction.

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Objective: The human gastro-oesophageal junction is exposed to abundant amounts of luminal reactive nitrogen-oxide species (RNOS) derived from the entero-salivary re-circulation of dietary nitrate. The aim of this study is to investigate the direct effects of luminal RNOS on the adjacent gastric barrier function using an ex vivo chamber model. Design and Setting: A chamber model in which the rat gastric mucosal membrane was mounted between the two halves of a chamber was designed to simulate the microenvironment of the lumen and the adjacent mucosa of the gastro-oesophageal junction. On the mucosal side of the chamber, RNOS was generated by the acidification of physiologic concentrations of sodium nitrite. The epithelial barrier function was evaluated by electrophysiological transmenbrane resistance and membrane permeability with 3H-mannitol flux. The expression of occludin was evaluated by immunohistochemistry and immunoblotting. Dinitrosyl dithiolato iron complex (DNIC) was also measured by means of electron paramagnetic resonance spectroscopy to confirm the diffusion of RNOS from the mucosal lumen into the mounted mucosa. Results: The administration of acidified nitrite to the mucosal lumen caused both a decrease in transmenbrane resistance and increase in epithelial permeability, suggesting a disturbance of the gastric barrier function. These changes were accompanied by a derangement of the expression of occludin. The diffusion of luminal RNOS into the mounted membrane was confirmed by showing the generation of DNIC within the tissue. Conclusions: Simulating the microenvironment of the human gastro-oesophageal junction, this study demonstrated that RNOS generated luminally at the human gastro-oesophageal junction can derange the barrier function of the adjacent tissue by disrupting the tight junction.

53: Behav Brain Res 2008 Jun;

Dose dependence and therapeutic window for the neuroprotective effects of curcumin in thromboembolic model of rat.

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Curcumin (diferuloylmethane), an active ingredient of turmeric, obtained from the powdered rhizomes of Curcuma longa Linn., has been traditionally recognized for treatment of several diseases. To evaluate the potential clinical use of curcumin, we determined the dose dependence of its effects in the therapeutic window and of the neuroprotective efficacy in a cerebral thromboembolic model of the rat. Rats were subjected to occlusion of the middle cerebral artery (MCAo) by a thrombus and treated with different doses of curcumin or the vehicle at 4h after ischemia. The animals were assessed after 24h for motor performance and neurological deficit. The rats were sacrificed immediately afterwards for evaluation of infarct, edema volume, estimation of nitrate and nitrite levels, neutrophil infiltration and levels of GSH and glutathione peroxidase (GSH-Px) in brain tissue. Curcumin reduced in a dose-dependent manner the ischemia-induced cerebral infarct and edema volume and attenuated neurological deficits observed after 24h. Curcumin reduced post-ischemic brain neutrophil infiltration, nitrate and nitrite levels and ameliorated the loss of GSH-Px and tends to increase the GSH levels but not significantly in the brain tissue. Neuronal levels of reactive oxygen species, peroxynitrite, and nitric oxide were lowered and in brain cryosections inducible nitric oxide synthase expression were significantly inhibited after treatment with curcumin. The present study is the first evidence of effectiveness of curcumin when given 4h post-ischemia in the rat thromboembolic stroke models, as it reduces infarct volume, ameliorates the sensory motor function and significantly attenuated the nitrosative stress.

54: Diabetologia 2008 Sep;

Inducible nitric oxide synthase gene deficiency counteracts multiple manifestations of peripheral neuropathy in a streptozotocin-induced mouse model of diabetes.

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AIMS/HYPOTHESIS: Evidence for the importance of peroxynitrite, a product of superoxide anion radical reaction with nitric oxide, in peripheral diabetic neuropathy is emerging. The role of specific nitric oxide synthase isoforms in diabetes-associated nitrosative stress and nerve fibre dysfunction and degeneration remains unknown. This study evaluated the contribution of inducible nitric oxide synthase (iNOS) to peroxynitrite injury to peripheral nerve and dorsal root ganglia and development of peripheral diabetic neuropathy. METHODS: Control mice and mice with iNos (also known as Nos2) gene deficiency (iNos (-/-)) were made diabetic with streptozotocin, and maintained for 6 weeks. Peroxynitrite injury was assessed by nitrotyrosine and poly(ADP-ribose) accumulation (immunohistochemistry). Thermal algesia was evaluated by paw withdrawal, tail-flick and hot plate tests, mechanical algesia by the Randall-Selitto test, and tactile allodynia by a von Frey filament test. RESULTS: Diabetic

wild-type mice displayed peroxynitrite injury in peripheral nerve and dorsal root ganglion neurons. They also developed motor and sensory nerve conduction velocity deficits, thermal and mechanical hypoalgesia, tactile allodynia and approximately 36% loss of intraepidermal nerve fibres. Diabetic iNos (-/-) mice did not display nitrotyrosine and poly(ADP-ribose) accumulation in peripheral nerve, but were not protected from nitrosative stress in dorsal root ganglia. Despite this latter circumstance, diabetic iNos (-/-) mice preserved normal nerve conduction velocities. Small-fibre sensory neuropathy was also less severe in diabetic iNos (-/-) than in wild-type mice. CONCLUSIONS/INTERPRETATION: iNOS plays a key role in peroxynitrite injury to peripheral nerve, and functional and structural changes of diabetic neuropathy. Nitrosative stress in axons and Schwann cells, rather than dorsal root ganglion neurons, underlies peripheral nerve dysfunction and degeneration.

55: Eur J Pharmacol 2008 Jun;

Therapeutic potential of 20(S)-ginsenoside Rg(3) against streptozotocin-induced diabetic renal damage in rats.

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The inhibitors of advanced glycation endproduct and oxidative stress, as well as N-methyl-d-aspartate (NMDA) receptor antagonists have received considerable interest because of their close association with renoprotective effects. The therapeutic potential of 20(S)-ginsenoside Rg(3) (20(S)-Rg(3)), isolated from Panax ginseng, against streptozotocin-induced diabetic renal damage, was investigated in this study. The diabetic rats received 5, 10, and 20 mg/kg body weight/day of 20(S)-Rg(3) orally via gavage for fifteen consecutive days. The physiological abnormalities such as increases in water intake and urine volume of diabetic rats were significantly decreased by the 20 mg/kg body weight of 20(S)-Rg(3) administration. The elevated serum glucose, glycosylated protein, and thiobarbituric acid-reactive substance levels in diabetic rats were also significantly reduced by the 20(S)-Rg(3) administrations. Moreover, the renal dysfunction of diabetic rats was significantly ameliorated by the 20(S)-Rg(3) administrations in a dose-dependent manner. These beneficial effects on diabetic renal damage were related to the inhibitory effect of 20(S)-Rg(3) against NMDA receptor-mediated nitrosative stress.

56: Arch Biochem Biophys 2008 Apr;

S-Adenosyl-l-methionine decreases the elevated hepatotoxicity induced by Fas agonistic antibody plus acute ethanol pretreatment in mice.

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The current study was designed to investigate the effect and potential mechanism of exogenous administration of S-adenosyl-l-methionine (SAM) on the enhanced hepatotoxicity induced by the Fas agonistic Jo2 antibody plus acute ethanol pretreatment in C57BL/6 mice. Acute ethanol plus Jo2 treatment produces liver toxicity under conditions in which ethanol alone

or Jo2 alone do not. SAM significantly attenuated this elevated hepatotoxicity in mice as manifested by a decrease of serum aminotransferases and morphological amelioration. Levels of SAM and activity of methionine adenosyltransferase were lowered by the ethanol plus Jo2 treatment but restored by administration of SAM. The ethanol plus Jo2 treatment increased activity and content of CYP2E1, iNOS content and TNF-alpha levels; these increases were blunted by SAM. SAM also protected against the elevated oxidative and nitrosative stress found after ethanol plus Jo2, likely due to the decreases in CYP2E1, iNOS and TNF-alpha. Calcium-induced swelling of mitochondria was enhanced by the ethanol plus Jo2 treatment and this was prevented by SAM. JNK and P38 MAPK were activated by the ethanol plus Jo2 treatment; JNK activation was partially prevented by SAM. It is suggested that SAM protects against the ethanol plus Jo2 toxicity by restoring hepatic SAM levels, preventing the increase in iNOS, CYP2E1 and TNF-alpha and there by lowering the elevated oxidative/nitrosative stress and activation of the JNK signal pathway, ultimately preventing mitochondrial damage.

57: Nitric Oxide 2008 Apr;

Molecular mechanisms for discrete nitric oxide levels in cancer.

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Nitric oxide (NO) has been invoked in nearly every normal and pathological condition associated with human physiology. In tumor biology, nitrogen oxides have both positive and negative affects as they have been implicated in both promoting and preventing cancer. Our work has focused on NO chemistry and how it correlates with cytotoxicity and cancer. Toward this end, we have studied both concentration- and time-dependent NO regulation of specific signaling pathways in response to defined nitrosative stress levels that may occur within the tumor microenvironment. Threshold levels of NO required for activation and stabilization of key proteins involved in carcinogenesis including p53, ERK, Akt and HIF have been identified. Importantly, threshold NO levels are further influenced by reactive oxygen species (ROS) including superoxide, which can shift or attenuate NO-mediated signaling as observed in both tumor and endothelial cells. Our studies have been extended to determine levels of NO that are critical during angiogenic response through regulation of the anti-angiogenic agent thrombospondin-1 (TSP-1) and pro-angiogenic agent matrix metalloproteinase-9 (MMP-9). The quantification of redox events at the cellular level has revealed potential mechanisms that may either limit or potentiate tumor growth, and helped define the positive and negative function of nitric oxide in cancer.

58: Transplant Proc 2008 Sep;40(7):2185-7

Restrictive ventilatory insufficiency and lung injury induced by ischemia/reperfusion of the pancreas in rats.

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BACKGROUND: Ischemia/reperfusion injury (I/R) of the rat pancreas induces acute pancreatitis with a systemic inflammatory response syndrome. Activated inflammatory cells sequestered in the lung and the proteases released from the inflammatory pancreas both induce acute lung injury. MATERIALS AND METHODS: Ischemia was induced by clamping the gastroduodenal artery and the splenic artery for 2 hours to induce ischemia of the pancreas, followed by reperfusion for 6 hours. We then observed lung function parameters, such as weight changes, compliance, functional residual capacity (FRC), and respiratory work. RESULTS: This protocol resulted in elevation in the blood concentrations of nitric oxide ($P < .05$), hydroxyl radicals ($P < .01$), amylase ($P < .05$), and white blood cells ($P < .001$) among the I/R group. Pulmonary function data showed that I/R of the pancreas induced significant decreases in the FRC and lung compliance (Cchord), but significant increases in respiratory work. The lung weight/body weight ratio also increased significantly. CONCLUSIONS: I/R of the pancreas induced lung injury and restrictive ventilatory insufficiency. Inflammatory responses in the lung tissues induced by oxidative stress and nitrosative stress may be major factors inducing lung injury and a restrictive type of ventilatory insufficiency.

59: Transplant Proc 2008 Sep;40(7):2156-8

Rat liver ischemia/reperfusion induced proinflammatory mediator and antioxidant expressions analyzed by gene chips and real-time polymerase chain reactions.

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OBJECTIVE: Ischemia/reperfusion (I/R) of the rat liver induces injury; however, few studies have investigated gene expressions associated with this phenomenon. In this study, gene chip and real-time polymerase chain reactions (PCR) were used to study the expressions of the proinflammatory mediators and antioxidants after I/R. MATERIALS AND METHODS: Ischemia was induced by clamping the common hepatic artery and portal vein for 40 minutes followed by 90 minutes reperfusion. Blood samples collected before ischemia and after reperfusion were analyzed for alanine amino transferase, lactic dehydrogenase, hydroxyl radicals, nitric oxide (NO), and tumor necrosis factor alpha (TNFalpha). Expressions of TNFalpha, interleukin 12 (IL12), cyclooxygenase II (COXII), and other inflammatory mediators were analyzed by gene chips. COXII, TNFalpha, and antioxidants of mitochondrial superoxide dismutase (SOD(Mn)), catalase, and heat shock protein 70 (HSP70) were double confirmed by real-time PCRs. RESULTS: This protocol resulted in elevations in the blood concentrations of NO, hydroxyl radicals, TNFalpha, ALT, and LDH ($P < .01$) in the I/R but not the sham-operated group. Reperfusion induced significant increases in the expressions of TNFalpha, IL12, COXII, SOD(Mn), catalase, and HSP70. Real-time PCR also demonstrated increases in mRNA expressions of the proinflammatory mediators and antioxidants. CONCLUSIONS: This protocol resulted in oxidative stress, nitrosative stress, and liver injury. The increases in expressions of both proinflammatory mediators and antioxidants suggested that an imbalance between inflammation and anti-inflammation could be the possible reason for the liver injury after I/R.

60: Naunyn Schmiedebergs Arch Pharmacol 2008 Aug;

Ruthenium red protects HepG2 cells overexpressing CYP2E1 against acetaminophen cytotoxicity.

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We studied the mechanisms of acetaminophen (APAP) cytotoxicity in HepG2 cells overexpressing cytochrome p4502E1, particularly the role of oxidative/nitrosative stress and ryanodine Ca(2+) channel. Cells were grown for 24 h with APAP in the presence or absence of 4-methylpyrazole (4MP), L:-arginine methyl ester (L-NAME), superoxide dismutase (SOD), or ruthenium red (RuR). Drug cytotoxicity was also tested in cells pretreated overnight with V-PYRRO/NO. APAP was without effect on empty vector-transfected cells, but damaged CYP2E1-transfected cells and this was abolished by RuR, reduced by 4MP, or V-PYRRO/NO but affected by L-NAME or SOD. APAP increased microsomal [(3)H]-ryanodine binding, while microsomal Ca(2+) uptake was significantly lowered. RuR increased net microsomal Ca(2+) uptake and normalized cytosolic Ca(2+) levels. We can conclude that neither oxidative nor nitrosative stress is relevant to APAP cytotoxicity in cultured HepG2 cells, but our results point to ryanodine receptors as a potential crucial protein in the early stages of APAP cytotoxicity.

61: Neurobiol Dis 2008 Aug;

Blood-brain barrier tight junction permeability and ischemic stroke.

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The blood-brain barrier (BBB) is formed by the endothelial cells of cerebral microvessels, providing a dynamic interface between the peripheral circulation and the central nervous system. The tight junctions (TJs) between the endothelial cells serve to restrict blood-borne substances from entering the brain. Under ischemic stroke conditions decreased BBB TJ integrity results in increased paracellular permeability, directly contributing to cerebral vasogenic edema, hemorrhagic transformation, and increased mortality. This loss of TJ integrity occurs in a phasic manner, which is contingent on several interdependent mechanisms (ionic dysregulation, inflammation, oxidative and nitrosative stress, enzymatic activity, and angiogenesis). Understanding the inter-relation of these mechanisms is critical for the development of new therapies. This review focuses on those aspects of ischemic stroke impacting BBB TJ integrity and the principle regulatory pathways, respective to the phases of paracellular permeability.

64: Antioxid Redox Signal 2008 Aug;

NITRIC OXIDE IN HEALTH AND DISEASE OF THE NERVOUS SYSTEM.

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Nitric oxide (NO) is an important messenger molecule in a variety of physiological systems. NO, a gas, is produced from L-arginine by different isoforms of nitric oxide synthase (NOS) and serves many normal physiological purposes, such as promoting vasodilation of blood vessels and mediating communication between nervous system cells. In addition to its physiological actions, free radical activity of NO can cause cellular damage through a phenomenon known as nitrosative stress. Here, we review the role of NO in health and disease, focusing on its role in function and dysfunction of the nervous system. There is substantial evidence that NO plays a key role in most common neurodegenerative diseases and, although the mechanism of NO-mediated neurodegeneration remains uncertain, studies suggest several possibilities. NO has been shown to modify protein function by nitrosylation and nitrotyrosination, contribute to glutamate excitotoxicity, inhibit mitochondrial respiratory complexes, participate in organelle fragmentation, and mobilize zinc from internal stores. In this review, we discuss and analyze the evidence for each of these mechanisms in different neurodegenerative diseases and propose future directions for research of NOs role in neurodegeneration.

69: J Hepatol 2008 Jun;

Prevention of alcoholic fatty liver and mitochondrial dysfunction in the rat by long-chain polyunsaturated fatty acids.

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BACKGROUND/AIMS: We reported that reduced dietary intake of polyunsaturated fatty acids (PUFA) such as arachidonic (AA,20:4n6,omega-6) and docosahexaenoic (DHA,22:6n3,omega-3) acids led to alcohol-induced fatty liver and fibrosis. This study was aimed at studying the mechanisms by which a DHA/AA-supplemented diet prevents alcohol-induced fatty liver. METHODS: Male Long-Evans rats were fed an ethanol or control liquid-diet with or without DHA/AA for 9 weeks. Plasma transaminase levels, liver histology, oxidative/nitrosative stress markers, and activities of oxidatively-modified mitochondrial proteins were evaluated. RESULTS: Chronic alcohol administration increased the degree of fatty liver but fatty liver decreased significantly in rats fed the alcohol-DHA/AA-supplemented diet. Alcohol exposure increased oxidative/nitrosative stress with elevated levels of ethanol-inducible CYP2E1, nitric oxide synthase, nitrite and mitochondrial hydrogen peroxide. However, these increments were normalized in rats fed the alcohol-DHA/AA-supplemented diet. The number of oxidatively-modified mitochondrial proteins was markedly increased following alcohol exposure but significantly reduced in rats fed the alcohol-DHA/AA-supplemented diet. The suppressed activities of mitochondrial aldehyde dehydrogenase, ATP synthase, and 3-ketoacyl-CoA thiolase in ethanol-exposed rats were also recovered in animals fed the ethanol-DHA/AA-supplemented diet. **CONCLUSIONS: Addition of DHA/AA prevents alcohol-induced fatty liver and mitochondrial dysfunction in an animal**

model by protecting various mitochondrial enzymes most likely through reducing oxidative/nitrosative stress.

70: Mol Pharmacol 2008 Aug;

REACTIVE OXYGEN SPECIES MEDIATE p53 ACTIVATION AND APOPTOSIS INDUCED BY SODIUM NITROPRUSSIDE IN SH-SY5Y CELLS.

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Sodium nitroprusside (SNP) is a water soluble iron nitrosyl complex clinically used as a powerful vasodilator for treatment of hypertension and, in basic research, to mainly investigate the cytotoxic effects of nitrosative stress. Although nitric oxide (NO) is considered a pharmacologically active molecule, not all the biological effects of SNP are dependent on its NO moiety. In order to elucidate the molecular executioner(s) responsible for SNP cytotoxicity, this study determines the involvement of oxidative stress in p53 activation and apoptotic induction elicited by SNP in SH-SY5Y neuroblastoma cells. We demonstrate that pro-apoptotic activity of SNP is independent on NO production, because SNP and its two-day light exhausted compound (SNPex) trigger apoptosis at the same extent. We provide evidence for the occurrence of oxidative stress and oxidative damages during both SNP and SNPex exposure and demonstrate that iron-derived reactive oxygen species (ROS) are the genuine mediators of their cytotoxicity. We show that p53 is equally activated upon both SNP and SNPex treatments. Moreover, as demonstrated by siRNA experiments, we indicate its primary role in the induction of apoptosis, suggesting the ineffectiveness of NO in its engagement. The attenuation of p53 levels, obtained by oxy-radical scavengers, consistently with the recovery of cell viability and ROS decrease, demonstrate that SNP-mediated p53 activation is an event triggered by ROS and/or ROS-mediated damages. Altogether our results suggest that investigations on the physio-pathological effects of SNP should consider the role of ROS, other than NO, particularly in some conditions such as apoptotic induction and p53 activation.

71: J Neurochem 2008 Jul;

NFkappaB in the mechanism of ammonia-induced astrocyte swelling in culture.

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Astrocyte swelling and brain edema are major neuropathological findings in the acute form of hepatic encephalopathy (fulminant hepatic failure), and substantial evidence supports the view that elevated brain ammonia level is an important etiological factor in this condition. Although the mechanism by which ammonia brings about astrocyte swelling remains to be determined, oxidative/nitrosative stress and mitogen-activated protein kinases (MAPKs) have been considered as important elements in this process. One factor known to be activated by both oxidative stress and MAPKs is nuclear factor kappaB (NFkappaB), a transcription factor that activates many genes, including inducible nitric oxide synthase (iNOS). As the product of iNOS, nitric oxide (NO), is known to cause astrocyte swelling, we examined the potential involvement of NFkappaB in ammonia-induced astrocyte swelling. Western blot analysis of cultured astrocytes showed a significant increase in NFkappaB nuclear translocation (a measure of NFkappaB activation) from 12 h to 2 days after treatment with NH(4)Cl (5 mM). Cultures treated with

anti-oxidants, including superoxide dismutase, catalase, and vitamin E as well as the MAPKs inhibitors, SB239063 (an inhibitor of p38-MAPK) and SP600125 (an inhibitor of c-Jun N-terminal kinase), significantly diminished NFkappaB activation by ammonia, supporting a role of oxidative stress and MAPKs in NFkappaB activation. The activation of NFkappaB was associated with increased iNOS protein expression and NO generation, and these changes were blocked by BAY 11-7082, an inhibitor of NFkappaB. Additionally, ammonia-induced astrocyte swelling was inhibited by the NFkappaB inhibitors, BAY 11-7082 and SN-50, thereby implicating NFkappaB in the mechanism of astrocyte swelling. Our studies indicate that cultured astrocytes exposed to ammonia display NFkappaB activation, which is likely to be a consequence of oxidative stress and activation of MAPKs. NFkappaB activation appears to contribute to the mechanism of ammonia-induced astrocyte swelling, apparently through its up-regulation of iNOS protein expression and the subsequent generation of NO.

72: Life Sci 2008 Jun;

Lycopene attenuates diabetes-associated cognitive decline in rats.

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Diabetes-induced learning and memory impairment, characterized by impaired cognitive functions and neurochemical and structural abnormalities, involve direct neuronal damage caused by intracellular glucose. The present study was designed to investigate the effect of lycopene, a potent anti-oxidant and anti-inflammatory molecule, on cognitive functions, oxidative stress and inflammation in streptozotocin (STZ)-induced 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 peripheral 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.

74: Free Radic Biol Med 2008 Apr;

The chemical biology of nitric oxide: Implications in cellular signaling.

Thomas DD, Ridnour LA, Isenberg JS, Flores-Santana W, Switzer CH, Donzelli S, Hussain P, Vecoli C, Paolocci N, Ambs S, Colton CA, Harris CC, Roberts DD, Wink DA

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Nitric oxide (NO) has earned the reputation of being a signaling mediator with many diverse and often opposing biological activities. The diversity in response to this simple diatomic molecule comes from the enormous variety of chemical reactions and biological properties associated with it. In the past few years, the importance of steady-state NO concentrations has emerged as a key determinant of its biological function. Precise cellular responses are differentially regulated by specific NO concentration. We propose five basic distinct concentration levels of NO activity: cGMP-mediated processes ([NO]<1-30 nM), Akt phosphorylation ([NO] = 30-100 nM), stabilization of HIF-1alpha ([NO] = 100-300 nM), phosphorylation of p53 ([NO]>400 nM), and nitrosative stress (1 muM). In general, lower NO concentrations promote cell survival and proliferation, whereas higher levels favor cell cycle arrest, apoptosis, and senescence. Free radical interactions will also influence NO signaling. One of the consequences of reactive oxygen species generation is to reduce NO concentrations. This antagonizes the signaling of nitric oxide and in some cases results in converting a cell-cycle arrest profile to a cell survival profile. The resulting reactive nitrogen species that are generated from these reactions can also have biological effects and increase oxidative and nitrosative stress responses. A number of factors determine the formation of NO and its concentration, such as diffusion, consumption, and substrate availability, which are referred to as kinetic determinants for molecular target interactions. These are the chemical and biochemical parameters that shape cellular responses to NO. Herein we discuss signal transduction and the chemical biology of NO in terms of the direct and indirect reactions.

77: Gastroenterology 2008 Jun;

Oxidative Inactivation of Key Mitochondrial Proteins Leads to Dysfunction and Injury in Hepatic Ischemia Reperfusion.

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BACKGROUND & AIMS: Ischemia-reperfusion (I/R) is a major mechanism of liver injury following hepatic surgery or transplantation. Despite numerous reports on the role of oxidative/nitrosative stress and mitochondrial dysfunction in hepatic I/R injury, the proteins that are oxidatively modified during I/R damage are poorly characterized. This study was aimed at investigating the oxidatively modified proteins underlying the mechanism for mitochondrial dysfunction in hepatic I/R injury. We also studied the effects of a superoxide dismutase mimetic/peroxynitrite scavenger metalloporphyrin (MnTMPyP) on oxidatively modified proteins and their functions. METHODS: The oxidized and/or S-nitrosylated mitochondrial proteins from I/R-injured mouse livers with or without MnTMPyP pretreatment were labeled with biotin-N-maleimide, purified with streptavidin-agarose, and resolved by 2-dimensional gel electrophoresis. The identities of the oxidatively modified proteins were determined using mass spectrometric analysis. Liver histopathology, serum transaminase levels, nitrosative stress markers, and activities of oxidatively modified mitochondrial proteins were measured. RESULTS: Comparative 2-dimensional gel analysis revealed markedly increased numbers of oxidized and S-nitrosylated mitochondrial proteins following hepatic I/R injury. Many key mitochondrial enzymes involved in cellular defense, fat metabolism, energy supply, and chaperones were identified as being oxidatively modified proteins. Pretreatment with MnTMPyP attenuated the I/R-induced increased serum

transaminase levels, histologic damage, increased inducible nitric oxide synthase expression, and S-nitrosylation and/or nitration of various key mitochondrial proteins. MnTMPyP pretreatment also restored I/R-induced suppressed activities of mitochondrial aldehyde dehydrogenase, 3-ketoacyl-CoA thiolases, and adenosine triphosphate synthase. CONCLUSIONS: These results suggest that increased nitrosative stress is critically important in promoting S-nitrosylation and nitration of various mitochondrial proteins, leading to mitochondrial dysfunction with decreased energy supply and hepatic injury.

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78: Am J Med Sci 2008 Jun;335(6):431-8

Cardiac fibrogenesis following infarction in mice with deletion of inducible nitric oxide synthase.

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BACKGROUND:: Studies have shown that the absence of inducible nitric oxide synthase (iNOS) improves cardiac function and survival after myocardial infarction (MI). The responsible mechanisms, however, remain uncertain. Cardiac iNOS is significantly increased after MI, which is colocalized with fibrous tissue formation. Herein, we tested our hypothesis that iNOS is involved in the development of cardiac fibrosis. METHODS:: Wild-type and iNOS-knockout mice were subjected to MI by left coronary artery ligation. At week 1, 2, 3, and 4 post-MI, we addressed cardiac expression of profibrogenic mediator, growth of collagen-producing cells, collagen synthesis, and degradation. RESULTS:: In the infarcted myocardium of wild-type and iNOS-knockout mice, transforming growth factor (TGF)-beta1 expression was significantly increased, particularly in the early stage; myofibroblasts appeared and became abundant for over 4 weeks; matrix metalloproteinase-1 expression was low, whereas tissue inhibitor of matrix metalloproteinase-1 was significantly elevated; type-I collagen mRNA was significantly increased and collagen was continuously accumulated. In the noninfarcted myocardium, TGF-beta1 and type-I collagen mRNA levels as well as collagen volume were also elevated, but less evident than infarcted myocardium. However, there was no significant difference in cardiac TGF-beta1 expression, myofibroblast population, collagen synthesis/degradation, and collagen volume between wild-type and iNOS-knockout mice with MI. CONCLUSION:: The current study suggests that iNOS-induced nitric oxide production may not mediate cardiac fibrosis after MI. Thus, other mechanisms are involved in nitrosative stress-induced cardiac dysfunction after MI.

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79: Appl Environ Microbiol 2008 Apr;

Response of Mycobacterium tuberculosis hemoglobin promoters to in vitro and in vivo growth conditions.

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The success of Mycobacterium tuberculosis as one of the dreaded human pathogens lies in its ability to utilize different defense mechanisms in response to the varied environmental challenges during the course of its intracellular infection, latency and reactivation cycle. Truncated hemoglobins, trHbN and trHbO, are considered to play pivotal roles in its cellular metabolism during stress and hypoxia. To delineate the genetic regulation of the M. tuberculosis hemoglobins, transcriptional fusion of the promoters of glbN and glbO genes with green fluorescent protein were constructed and their response was monitored in M. smegmatis and M. tuberculosis H37Ra, exposed to environmental stresses in vitro and in M. tuberculosis H37Ra after in vivo growth inside macrophage. Promoter activity of glbN increased substantially during stationary phase and was nearly 3-3.5-fold higher than glbO promoter, which remained more or less constant during different growth phases of M. smegmatis as well as M. tuberculosis H37Ra. In both the mycobacterial hosts, glbN promoter activity was induced 1.5-2-fold by the general nitrosative stress inducer, nitrite as well as the NO releaser, sodium nitroprusside (SNP). The glbO promoter was more responsive to nitrite as compared to SNP, although the overall increase in its activity was at a much lower level in comparison to glbN promoter. Additionally, the glbN promoter remained insensitive to oxidative stress generated by H2O2 but the glbO promoter activity increased to nearly 1.5-fold under similar conditions suggesting that the trHb gene promoters are regulated differently under nitrosative and oxidative stress. In contrast, the transition metal-induced hypoxia enhanced activity of both glbN and glbO promoters at all growth phases, with glbO promoter being induced to approximately 2.3-fold, which was found highest for this promoter under all the conditions evaluated. The addition of iron along with nickel reversed the induction in both the cases. Interestingly, a concentration-dependent decrease in the activity of both trHb gene promoters was observed when the levels of iron were depleted in the growth media by addition of an iron chelator. These results suggested the involvement of an iron/heme-containing oxygen sensor in the modulation of the trHb gene promoter activities directly or indirectly in conjunction with other cellular factors. Mode of promoter regulation under different physiological conditions was found to be similar for the trHbs in both M. smegmatis and M. tuberculosis H37Ra indicating that these promoters might be regulated by components that are common to both the systems. Confocal microscopy of the THP-1 macrophages infected with M. tuberculosis carrying the trHb gene promoter fusions showed significant level of promoter activity during intracellular growth in macrophages. Time course evaluation of the promoter activity after various times points up to 48 hr through FACS analysis of the intracellular M. tuberculosis cells indicated the glbN promoter to be active at all time points assessed, whereas, the glbO promoter remained at a steady state level upto 24 hr post infection and increased by approximately 2-fold after 48 hr of infection. Overall regulation pattern of M. tuberculosis trHb gene promoters, thus, correlates not only with the stresses that tubercle bacillus is likely to encounter once inside the macrophage environment but also with the present day knowledge of their functionalities. The in vivo studies, demonstrating for the first time the expression of trHbs during macrophage infection of M. tuberculosis, strongly point towards the requirement and thus, the importance of these hemoglobins during intracellular regime of the bacterium. The present study conducted herein on transcriptional regulation of M. tuberculosis hemoglobins, in vitro under various stress conditions and in vivo after macrophage infection, substantiate that biosynthesis of both trHbs, trHbN and trHbO, in its native host is regulated via the environmental signals that tubercle bacillus faces during macrophage infection and growth in its human host.

80: Chest 2007 Nov;

Variation in iron homeostasis genes between patients with acute respiratory distress syndrome and healthy controls.

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Background Abnormal plasma and lung iron mobilization is associated with the onset and progression of ARDS and is detectable in specific at risk populations. Patients with ARDS also suffer pronounced oxidative and nitrosative stress which can be catalyzed, and thereby aggravated, by the bioavailability of redox active iron. ARDS of pulmonary and extrapulmonary origin may differ pathophysiologically and require different ventilatory strategies. Evidence suggests that genetic predisposition is relevant to the pathogenesis of ARDS. We therefore explored the hypothesis that polymorphisms from a panel of genes encoding iron metabolizing proteins determine susceptibility to ARDS. Methods Retrospective case-control study conducted at the adult intensive care units of two university hospitals. Patients with ARDS (n=122) and healthy controls (n=193) were genotyped. Sequence-specific primer PCR was used to genotype selected biallelic single nucleotide polymorphisms. An audit of the patient database was conducted and 104 of the 122 ARDS patients were eligible for the final data analysis. Results Preliminary analysis indicated differences between ARDS and healthy controls in the incidence of polymorphism of the gene encoding ferritin light chain. Subgroup analysis indicated the prevalence of ferritin light-chain gene -3381GG homozygotes was increased in patients with ARDS of extrapulmonary origin compared to healthy controls. Secondly, a common haplotype in the heme oxygenase 2 gene was reduced in patients with ARDS compared to healthy controls and was more evident in those with ARDS of direct, or pulmonary etiology. Conclusions These results provide preliminary evidence to suggest a distinction in the genetic background of the sub-populations studied, inferring that the ferritin light-chain gene genotype confers susceptibility to ARDS, whilst the heme oxygenase 2 haplotype is protective against the onset of the syndrome. Such data support further previous findings that suggest abnormalities in iron handling resulting in redox imbalance are implicated in the pathogenesis of ARDS.

81: Circ J 2008;72(6):998-1002

Effect of Pioglitazone on Nitroglycerin-Induced Impairment of Nitric Oxide Bioavailability by a Catheter-Type Nitric Oxide Sensor.

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Background We examined whether nitroglycerin (NTG)-induced impairment of nitric oxide (NO) bioavailability could be modified by a peroxisome proliferator-activated receptor (PPAR) gammaagonist. Methods and Results Male New Zealand White rabbits were treated for 7 days with NTG patches, either alone or in combination with pioglitazone. Plasma NO concentration was measured with the catheter-type NO sensor located in the aorta. N(G)-methyl-L-arginine and acetylcholine (ACh) were infused into the aortic arch to measure the basal and ACh-induced plasma NO concentrations. Vascular nitrotyrosine and tetrahydrobiopterin (BH(4)) concentrations were measured by enzyme-linked immunosorbent assay and high-performance liquid chromatography with fluorescence detection, respectively. The negative effects of NTG, that is, the decrease in basal and ACh-induced NO production, were significantly suppressed by co-treatment with pioglitazone. NTG-induced increases in vascular nitrotyrosine and BH(4) concentrations were significantly decreased with co-treatment with pioglitazone. Conclusions NTG-induced impairment of basal and ACh-stimulated NO production might be prevented by the co-treatment with a PPAR gamma agonist, pioglitazone through suppressions of nitrosative stress. (Circ J 2008; 72: 998 - 1002).

82: J Biol Inorg Chem 2008 Mar;

Iron-sulfur repair YtfE protein from Escherichia coli: structural characterization of the di-iron center.

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YtfE was recently shown to be a newly discovered protein required for the recovery of the activity of iron-sulfur-containing enzymes damaged by oxidative and nitrosative stress conditions. The Escherichia coli YtfE purified protein is a dimer with two iron atoms per monomer and the type and properties of the iron center were investigated by using a combination of resonance Raman and extended X-ray absorption fine structure spectroscopies. The results demonstrate that YtfE contains a non-heme dinuclear iron center having mu-oxo and mu-carboxylate bridging ligands and six histidine residues coordinating the iron ions. This is the first example of a protein from this important class of di-iron proteins to be shown to be involved in the repair of iron-sulfur centers.

83: Microbiology 2008 Jun;154(Pt 6):1763-74

Pleiotropic roles of iron-responsive transcriptional regulator Fur in Burkholderia multivorans.

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The fur (ferric uptake regulator) gene of Burkholderia multivorans ATCC 17616 was identified by transposon mutagenesis analysis. The fur deletion mutant of strain ATCC 17616 (i) constitutively produced siderophores, (ii) was more sensitive to reactive oxygen species (ROS) than the wild-type

strain, (iii) showed lower superoxide dismutase and catalase activities than the wild-type strain, (iv) was unable to grow on M9 minimal agar plates containing several substrates that can be used as sole carbon sources by the wild-type strain, and (v) was hypersensitive to nitrite and nitric oxide under microaerobic and aerobic conditions, respectively. These results clearly indicate that the Fur protein in strain ATCC 17616 plays pleiotropic roles in iron homeostasis, removal and/or resistance to ROS and nitrosative stress, and energy metabolism. Furthermore, employment of an in vivo Fur titration assay system led to the isolation from the ATCC 17616 genome of 13 Fur-binding DNA regions, and a subsequent electrophoretic mobility-shift assay confirmed the direct binding of Fur protein to all of these DNA regions. Transcriptional analysis of the genes located just downstream of the Fur-binding sites demonstrated that Fur acts as a repressor for these genes. Nine of the 13 regions were presumed to be involved in the acquisition and utilization of iron.

84: Epilepsia 2008 May;

Glutamine synthetase becomes nitrated and its activity is reduced during repetitive seizure activity in the pentylentetrazole model of epilepsy.

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Purpose: The astrocyte-specific glutamine synthetase (GS) plays a key role in glutamate recycling and Gamma-aminobutyric acid (GABA)metabolism. Changes in the expression or activity of GS have been proposed to contribute to epileptogenesis. The mechanisms or how and where GS may contribute to epilepsy is still a matter of discussion. Here we asked the question whether brain regions, which show an astrocytic stress response respond with alterations of GS. Methods: Biochemical and histological alterations of GS, HSP-27, and GFAP were studied after pentylenetetrazole-induced repetitive epileptic seizures (PIRS) in rats using a topographical quantification of the GS-immunoreactivity (GSIR) in relation to the focal heat shock response (HSR). Saline-treated rats served as controls and rats treated by the GS-inhibitor, L-methionine-sulfoximine (MSO) served as a positive control. Results: No changes in the amount of GSIR and GS-protein occurred during PIRS. A significant reduction of GSIR was observed by histochemistry (in situ) and in native (nonheated) protein extracts of MSO-treated rats. In rats affected by PIRS, GS-activity showed a significant, region-specific reduction in association with a nitration of the enzyme. Discussion: These results show that neither PIRS nor GS-inhibition reduced the amount of GS protein, but that MSO interferes with antibody binding to native GS. PIRS resulted in a focal increase of astrocytic stress response, whereas MSO caused a widespread, homogeneous astrocytic HSR independent from quantitative changes of GS content. In rats with PIRS the regions showing a strong glial HSR, respond with reduced GS-activity and GS-nitration, which all together are clear indicators of a nitrosative stress response.

85: Cell Death Differ 2008 Feb;

Secretogranin II: a key AP-1-regulated protein that mediates neuronal differentiation and protection from nitric oxide-induced apoptosis of neuroblastoma cells.

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Identification of AP-1 target genes in apoptosis and differentiation has proved elusive. Secretogranin II (SgII) is a protein widely distributed in nervous and endocrine tissues, and abundant in neuroendocrine granules. We addressed whether SgII is regulated by AP-1, and if SgII is involved in neuronal differentiation or the cellular response to nitrosative stress. Nitric oxide (NO) upregulated sgII mRNA dependent on a cyclic AMP response element (CRE) in the sgII promoter, and NO stimulated SgII protein secretion in neuroblastoma cells. Upregulation of sgII mRNA, sgII CRE-driven gene expression and SgII protein synthesis/export were attenuated in cells transformed with dominant-negative c-Jun (TAM67), which became sensitized to NO-induced apoptosis and failed to undergo nerve growth factor-dependent neuronal differentiation. Stable transformation of TAM67 cells with sgII restored neuronal differentiation and resistance to NO. RNAi knockdown of sgII in cells expressing functional c-Jun abolished neuronal differentiation and rendered the cells sensitive to NO-induced apoptosis. Therefore, SgII represents a key AP-1-regulated protein that counteracts NO toxicity and mediates neuronal differentiation of neuroblastoma cells.Cell Death and Differentiation advance online publication, 1 February 2008; doi:10.1038/cdd.2008.8.

86: Curr Opin Gastroenterol 2008 May;24(3):328-38

Alcohol and the liver.

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PURPOSE OF REVIEW: To update the reader with advances in epidemiology, genetics, detection, pathogenesis and therapy of alcohol-related liver disease. RECENT FINDINGS: Ill-health due to alcohol abuse is improving in some nations but deteriorating in others. Oxidative and nitrosative stress are key to the pathogenesis of alcoholic liver disease, and there is now greater emphasis than previously on their development and role of cytochrome P450 2E1, on mitochondrial stress and disruption, (including elucidation of mitochondrial protection mechanisms) disturbance of signaling pathways and involvement of extrahepatic mediators like adiponectin. Treatment of alcoholic liver disease has stagnated, but transplantation is still favored and debated for end-stage cirrhosis. SUMMARY: Basic and clinical research into the mechanisms of alcoholic liver disease is making headway, but has yet to produce safe and effective therapies for alcoholic hepatitis and for reversing cirrhosis.

87: J Clin Biochem Nutr 2008 May;42(3):175-84

Inflammatory response in microvascular endothelium in sepsis: role of oxidants.

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Sepsis, as a severe systemic inflammatory response to bacterial infection, represents a major clinical problem. It is characterized by the excessive production of reactive oxygen species (ROS) both in the circulation and in the affected organs. The excessive generation of ROS inevitably leads to oxidative stress in the microvasculature and has been implicated as a causative event in a number of pathologies including sepsis. In this review, we focus on the role of oxidative and nitrosative stress during the early onset of sepsis. Changes in microvascular endothelial cells, the cell type that occurs in all organs, are discussed. The mechanisms underlying septic induction of oxidative and nitrosative stresses, the functional consequences of these stresses, and potential adjunct therapies for microvascular dysfunction in sepsis are identified.

88: J Neuropathol Exp Neurol 2008 May;67(5):417-427

Trauma-Induced Cell Swelling in Cultured Astrocytes.

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Brain edema and associated increased intracranial pressure are major consequences of traumatic brain injury that account for most early deaths after traumatic brain injury. An important component of brain edema after traumatic brain injury is astrocyte swelling (cytotoxic edema). To examine the pathophysiologic mechanisms of trauma-induced astrocyte swelling, we used an in vitro fluid percussion trauma model. Exposure of cultured rat astrocytes to 5 atm of pressure resulted in significant cell swelling at 1 to 24 hours posttrauma that was maximal at 3 hours. Because oxidative/nitrosative stress, mitochondrial permeability transition (mPT), and mitogen-activated protein kinases (MAPKs) have been implicated in astrocyte swelling in other neurologic conditions, we examined their potential roles in this model. We previously showed increased free radical generation after in vitro trauma and show here that trauma to astrocytes increased the production of nitric oxide. Trauma also induced mPT and increased phosphorylation (activation) of MAPKs (extracellular signal-regulated kinase 1/2, c-Jun-N-terminal kinase, and p38-MAPK); these changes were diminished by antioxidants and the nitric oxide synthase inhibitor N-nitro-l-arginine methyl ester. Antioxidants, N-nitro-l-arginine methyl ester, the mPT inhibitor cyclosporin A, and inhibitors of MAPKs all significantly diminished trauma-induced astrocyte swelling. These findings demonstrate that direct mechanical injury to cultured astrocytes brings about cell swelling, and that blockade of oxidative/nitrosative stress, mPT, and MAPKs significantly reduce such swelling.

89: J Endocrinol 2008 Apr;197(1):139-50

Interaction between pro-inflammatory and anti-inflammatory cytokines in insulin-producing cells.

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Pro-inflammatory cytokines cause beta-cell dysfunction and death. The aim of this study was to investigate the interactions between different pro- and anti-inflammatory cytokines and their effects on apoptotic beta-cell death pathways. Insulin-producing RINm5F cells were exposed to different combinations of cytokines. Gene expression analyses of manganese superoxide dismutase (MnSOD) and inducible nitric oxide synthase (iNOS) were performed by real-time RT-PCR. Cell viability was measured by the MTT assay, NFkappaB activation using a SEAP reporter gene assay, protein expression by western blotting and caspase-3 activity using the DEVD cleavage method. IL-1beta, tumour necrosis factor alpha (TNFalpha) and a combination of all three pro-inflammatory cytokines increased while IFNgamma alone did not affect NFkappaB activity and iNOS gene and protein expression. Interestingly, the anti-inflammatory cytokines IL-4, IL-13 and IL-10 decreased IL-1beta-stimulated NFkappaB activation and iNOS expression. IL-1beta, TNFalpha and the pro-inflammatory cytokine combination also increased MnSOD gene and protein expression. But IL-4, IL-13 and IL-10 did not affect MnSOD expression and did not modulate IL-1beta-stimulated MnSOD expression. Caspase-3 activity was increased by IL-1beta and the pro-inflammatory cytokine combination, and to a lesser extent by TNFalpha. In contrast, IFNgamma had no effect on caspase-3 activity. IL-4, IL-13 and IL-10 decreased caspase-3 activity and increased viability of insulin-producing cells treated with pro-inflammatory cytokines. The anti-inflammatory cytokines counteracted the cytotoxic effects of pro-inflammatory cytokines in insulin-producing cells. This was achieved through the reduction of nitrosative stress. Thus, a balance between the anti-inflammatory and the pro-inflammatory cytokines is of crucial importance for the prevention of pancreatic beta-cell destruction.

93: Hypertension 2008 Jan;

Addition of Eplerenone to an Angiotensin-Converting Enzyme Inhibitor Effectively Improves Nitric Oxide Bioavailability.

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Angiotensin II and aldosterone both promote endothelial dysfunction and atherosclerosis. We investigated the effect of a combination of eplerenone, a selective aldosterone antagonist, and enalapril, an angiotensin-converting enzyme inhibitor, on NO bioavailability and spontaneous atherosclerotic changes. Twenty-four myocardial infarction-prone Watanabe heritable hyperlipidemic rabbits were treated with vehicle (control), eplerenone (50 mg/kg per day), enalapril (3 mg/kg per day), or eplerenone plus enalapril for 8 weeks (n=6 in each group). After treatment,

acetylcholine-induced NO production was measured as a surrogate for endothelium-protective function, and vascular peroxynitrite (a product of superoxide and NO) was measured to assess dysfunctional endothelial NO synthase activity. Plaque area was quantified by histology. Intra-aortic infusion of acetylcholine produced an increase in plasma NO concentration that was significantly higher with all of the drug treatments compared with the control. Eplerenone and enalapril, in combination, increased acetylcholine-induced NO by 7.9 nM, which was significantly higher than with either eplerenone or enalapril alone. Vascular peroxynitrite was significantly higher in the control group (1.3 pmol/mg of protein) and significantly lower with combination treatment (0.4 pmol/mg of protein) compared with the enalapril or eplerenone group. The highest tetrahydrobiopterin levels were observed after cotreatment with eplerenone and enalapril. Histology of the thoracic aorta showed a significantly decreased plaque area with combination therapy compared with monotherapy. Combined treatment with a selective aldosterone antagonist and an angiotensin-converting enzyme inhibitor has additive protective effects on endothelial function and on atherosclerotic changes via decreased nitrosative stress.

94: J Neurochem 2007 Nov;

Identification of Poly-ADP-Ribosylated Mitochondrial Proteins after Traumatic Brain Injury.

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Poly-ADP-ribosylation is a post-translational modification performed by poly(ADP-ribose) polymerases (PARP), involved in many diverse cellular functions including DNA repair, transcription, and long-term potentiation. Paradoxically, PARP over-activation under pathologic conditions including traumatic brain injury (TBI) results in cell death. We previously demonstrated that intra-mitochondrial poly-ADP-ribosylation occurs following excitotoxic and oxidative injury in vitro. Here we sought to identify mitochondrial proteins modified by poly-ADP-ribosylation after TBI in vivo. Poly-ADP-ribosylation within mitochondria from injured brain after experimental TBI in rats was first verified using western blot and immuno-electron microscopy. Poly-ADP-ribosylated mitochondrial proteins identified using a targeted proteomic approach included voltage-dependent anion channel-1, mitofilin, mitochondrial stress proteins, and the electron transport chain components F(1)F(0) ATPase, cytochrome c oxidase, and cytochrome c reductase. To examine the functional consequences of mitochondrial poly-ADP-ribosylation, isolated rat brain mitochondria were exposed to conditions of nitrosative stress known to activate PARP. PARP activation-induced reductions in State 3 respiration were prevented by the PARP-1 inhibitor 5-iodo-6-amino-1,2-benzopyrone or exogenous poly(ADP-ribose) glycohydrolase (PARG). Since the effects of PARP activation on mitochondrial respiration appear regulated by PARG, a direct effect of poly-ADP-ribosylation on electron transport chain function is suggested. These findings may be of relevance to TBI and other diseases where mitochondrial dysfunction occurs.

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95: Chem Biol Interact 2007 Nov;

The tandem of free radicals and methylglyoxal.

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Methylglyoxal is an alpha-oxoaldehyde inevitably produced from triose-phosphate intermediates of phosphorylating glycolysis, and also from amino acids and acetone. Recently, the attention has been focused on the involvement of free radicals in methylglyoxal toxicity. In this review, a summary of the relationship between methylglyoxal metabolism and free radical production is presented, extending discussion from the possible metabolic routes to the toxicological events by reviewing the role of free radicals in both generation and degradation of this 1,2-dicarbonyl as well as in the modification of biological macromolecules, and focusing on the action of methylglyoxal upon cellular glutathione content. Methylglyoxal-provoked free radical generation involving reactive oxygen species (ROS), reactive nitrogen species (RNS) as well as organic radicals like methylglyoxal radial or crosslinked protein radical as potential risk factors to tissue damage propagation, is thoroughly discussed. Special attention is paid to the potential therapeutic interventions. The paper arrives at the conclusion that a tight junction exists between methylglyoxal toxicity and free radical (particularly ROS) generation, though the toxicity of 1,2-dicarbonyl evolves even under anaerobic conditions, too. The events follow a sequence beginning with carbonyl stress essential for the toxicity, leading to free radical formation and finally ending in either apoptosis or necrosis. Both oxidative and nitrosative stress play important but not indispensable role in the development of methylglyoxal toxicity.

100: Free Radic Biol Med 2008 Jun;

Blockade of neuronal nitric oxide synthase reduces cone cell death in a model of retinitis pigmentosa.

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Retinitis pigmentosa (RP) is a group of diseases in which many different mutations cause rod photoreceptor cells to die and then gradually cone photoreceptors die due to progressive oxidative damage. In this study, we have shown that peroxynitrite-induced nitrosative damage also occurs. In the rd1 mouse model of RP, there was increased staining for S-nitrosocysteine and nitrotyrosine protein adducts that are generated by peroxynitrite. Peroxynitrite is generated from nitric oxide (NO) and superoxide radicals. After degeneration of rods, injection of hydroethidine resulted in strong fluorescence in the retina of rd1 mice, indicating high levels of superoxide radicals, and this was reduced, as was nitrotyrosine staining, by apocynin, suggesting that overaction of NADP(H) oxidase is at least partially responsible. Treatment of rd1 mice with a mixture of nitric oxide synthase (NOS) inhibitors markedly reduced S-nitrosocysteine and nitrotyrosine staining and significantly increased cone survival, indicating that NO-derived peroxynitrite contributes to cone cell death.

Treatment with 7-nitroindazole, a relatively specific inhibitor of neuronal NOS, also significantly reduced cone cell death, but aminoguanidine, a relatively specific inhibitor of inducible NOS, did not. These data suggest that NO generated by neuronal NOS exacerbates oxidative damage to cones in RP and that combined therapy to reduce NO and oxidative stress should be considered.