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Poster Presentation Abstracts

PP1

A New Protocol for the Evaluation of the Antioxidant Activity of Plant Components

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A new approach is proposed to evaluate the antioxidant activity. It is based on simultaneous measurement of cellular turbidity and hemoglobin. Human erythrocytes were pretreated separately with ginger oleoresin, ginger essential oil, and ascorbic acid. Untreated cells served as control. Oxidative stress was induced by H_2O_2. Samples were then evaluated by simultaneous measurement of cellular turbidity and the released hemoglobin. Additionally, morphological changes of erythrocytes, catalase activity, and lipid peroxydation were investigated. The results showed that, paradoxically, hemoglobin was significantly higher in samples treated with ginger extracts compared to the control. Surprisingly, cell concentrations were also higher in these same samples. This means that, when working under antioxidant conditions, hemoglobin alone is not an indicator of hemolysis. These findings were supported by the measurement of catalase activity and lipid peroxidation. In conclusion, hemolysis test should be performed by concurrent measurement of erythrocyte concentration and hemoglobin when working under antioxidant conditions.

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PP2

Modulation of oxidative stress in human hippocampal progenitor cells: a model to study underlying mechanisms of depression

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Background: Recent findings suggest that oxidative stress (OS) plays an important role in the pathophysiology of depression. Excessive reactive oxygen species (ROS) alter neural cytoarchitecture and function and could contribute to lower neuroplasticity and reduced neurogenesis, both of which have been linked to lower mood. This study aimed to explore the effects of OS on human hippocampal progenitor cells capable of neurogenesis.

Methods: We induced OS in human hippocampal progenitor cells by treatment with tert-butylhydroperoxide (T-BHP) in different concentrations. Cell viability was assessed by the MTS assay. Level of malondialdehyde (MDA) was measured in cells supernatant using the TBARS assay. Activation of Nf-kB and Nrf2 transcription factors were followed by immunostaining and TransAM kit respectively. After differentiation cells were labeled with DCX and MAP2 – markers of young and mature neurons and assessed using CellInsight platform.

Results: At 100 μM, 200 μM and 500 μM, T-BHP caused a reduction of cell viability for 23%, 54%, and 74% respectively, compared with vehicle, with a concomitant increase in levels of MDA. At 50 μM and 100 μM of T-BHP, Nf-κB positive cells increased by 16% and 17% respectively, while Nrf2 dose-dependently translocated to the nucleus. At 1 μM, 10 μM and 25 μM, T-BHP increased MAP2 and DCX positive cells by 34%, 33% and 24% for MAP2 and 38% and 30% for at 1 μM and 10 μM for DCX.

Conclusions: Our results show that cell damage and cell death of human hippocampal progenitor cells caused by T-BHP occur in a dose-dependent manner. We found activation of transcription factors Nf-κB and Nrf2 in response to OS stimuli. Our results also indicate higher levels of neurogenesis in cells treated with low doses of T-BHP. Thus, we conclude that ROS serve as secondary messengers and facilitate various signaling pathways regulating fundamental neurobiological processes known to be affected in depression.

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PP3

Role of oxidation and thermal unfolding in structural changes to beta-lactoglobulin

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Oxidation is a major cause of protein deterioration in mammals, plants, foodstuffs and pharmaceuticals, with this giving rise to changes in amino acid composition, fragmentation, aggregation, solubility, hydrophobicity, conformation, susceptibility to digestion and function. Whilst it is well established that these processes have negative impacts on product quality and human health, the mechanisms that generate these alterations are incompletely understood. In this project we are examining whether and how unfolding and oxidation induce changes in the structure of beta-lactoglobulin, to determine whether unfolding alters the rate and mechanism by which samples undergo oxidation, and vice versa. Gaining an understanding of how this occurs is critical to the development of methods to stop undesirable changes, particularly in dairy products where heat treatment is widely used, and oxidation is often encountered.

Heat treatment has been shown to result in unfolding of beta-lactoglobulin as assessed by circular dichroism measurements. This effect was increased, in a dose-dependent manner, in the presence of added H_2O_2. Addition of high doses of H_2O_2 before heating also resulted in a shift of the intrinsic (Trp-derived) protein fluorescence maximum to higher wavelengths. Thiol quantification, using 5,5'-dithio-(2-nitrobenzoic acid), revealed that H_2O_2 depletes thiols in a dose-dependent manner. Using SDS-PAGE, it was shown that whilst heating induced reducible cross-links; pretreatment with high levels of H_2O_2 prevented the formation of these species. With lower concentrations of H_2O_2, reducible cross-links were generated during subsequent heating, consistent with a key role for disulphide formation in heat-induced cross-link formation, with H_2O_2 diminishing aggregate formation. Altogether, these data indicate that both thermal treatment and oxidation can induce modifications to the protein via competing processes, and alter the vulnerability of the protein to aggregation.

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PP4

Carbon monoxide releasing molecule-3 (CORM-3) modulates progression of M1/M2 phenotypes in alveolar macrophages

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Background: The outcome of the severe systemic disorders (e.g. sepsis, acute lung injury) largely depends on the efficacy of resolution of inflammation. We have reported that water-soluble carbon monoxide releasing molecule-3 (CORM-3) suppresses inflammatory activation in neutrophils and vascular endothelial cells and offers protection against sepsis-induced lung dysfunction *in vivo*. Alveolar macrophages are key contributors to both the promotion and resolution/remodeling of inflammation and are classified into pro-inflammatory (M1) and anti-inflammatory (M2) groups. The change in M1/M2 balance has been reported in various pulmonary diseases and is considered as a target for therapeutic intervention. The aim of this study was to assess the effects of CORM-3 in modulation of macrophage M1/M2 phenotype under "inflammatory" or "remodeling" conditions.

Methods: Rat alveolar macrophages (NR8383) in culture were stimulated with LPS (500ng/ml)/IFN-γ (100ng/ml) or IL-4 (1ng/ml)/IL-13(1ng/ml) to induce M1- and M2-phenotypes, respectively. Specific markers of M1-phenotype (iNOS expression) and M2-phenotype (mannose receptor; Man-R, expression) were assessed (western blotting) after 1, 3, 6, or 24 hours in the absence or presence of CORM-3 (0.1mM) treatment. Inactive CORM-3 was used as a control.

Results: M1-phenotype (iNOS expression) was markedly upregulated (10 to 24 –fold) in LPS/IFN-γ-stimulated macrophage after 6 and 24 hours, respectively. In parallel, IL-4/IL13 stimulation induced M2-phenotype as evidenced by increase in Man-R levels at 6 and 24 hrs. Administration of CORM-3 during macrophage stimulation effectively suppressed development of both, M1- and M2-phenotypes. Paradoxically, treatment of naïve (unstimulated) macrophage with CORM-3 increased expression of Man-R after 1 hour (1.8 fold) and iNOS expression after 6 hours (2.5 fold).

Conclusion: These findings indicate that CORM-3 can modulate progression of M1- and M2-phenotypes in macrophages under inflammatory conditions *in vitro*.

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PP5

Radioprotection by expression of *Cryptosporidium parvum* thioredoxin peroxidase gene in mammalian cells

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Cryptosporidium parvum is one of the most radioresistant organisms identified to date, and thioredoxin peroxidase (CpTPx) is considered one of the molecules responsible for its radioresistance. To assess the potential of CpTPx to confer radioprotection in mammalian cells, it was expressed in African green monkey kidney cells (COS-7), to give CpTPx-COS-7 cells. The thioredoxin peroxidase of *Cryptosporidium muris* (CmTPx) was also expressed in COS-7 cells (CmTPx-COS-7 cells), which were used for comparative analysis with CpTPx-COS-7 cells. Survival rates of CpTPx-COS-7 cells, at 72 h after 8 Gy irradiation, were significantly higher (112-122%) than those of CmTPx-COS-7 or non-transfected COS-7 (ntCOS-7) cells. In addition, CpTPx showed stronger intracellular ROS scavenging effects in COS-7 cells, and gamma-H2AX expression and apoptotic changes after irradiation reduced compared to those observed in ntCOS-7 cells. CmTPx was shown to have antioxidant and DNA damage protection activities; however, these activities were always lower than those of CpTPx.These results suggest that the potent antioxidant and DNA damage protection activities of CpTPx were well conserved in this cell-based system and that CpTPx contributed to the radioprotection of mammalian cells through its exceptional antioxidant activity.

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PP6

Assessment of changes in biochemical indices of free radical oxidation in the semen of men living in the Aral Sea region

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Introduction: At present, the Aral Sea crisis is a global environmental problem, because negative trends in the state of the environment and changes in health outcomes have gained importance for Kazakhstan. In the Aral Sea region a lot of attention is paid to the effects on the body of heavy metals, pesticides, nitrates and other toxicants.

The aim of our study was to evaluate changes in biochemical parameters free radical in the semen of men living in the Aral Sea region.

Materials and methods: The study served as the sperm of men who live in areas of environmental disaster and environmental crisis, which were divided on three age groups: 1 group - 18 - 29 yrs, 2 group - 30 - 39 yrs, 3 group - 40 - 49 yrs. Determination of malondialdehyde (MDA) was performed by the modified method Korobeynikova Ye.N.(1989). Determination of average molecular method Kovalevskii A.N and Nifanteva O.E. (1990).

Results: The study found that in men of all age groups living in the township Shieli zone of ecological crisis level of MDA - in semen decreased relative performance in men living in the city of Aral zone of ecological disaster.

To identify toxicity cells was determined content of middle molecules, which are universal biochemical marker of endogenous intoxication in persons living in environmentally disadvantaged areas.

Established a significant increase in the level of middle molecules in the semen of men living in the area of environmental crisis, as compared with the men living in the region of ecological disaster.

Conclusion: In the zones of ecological crisis and ecological disaster in the men surveyed were found changes in the level of secondary metabolites of lipid peroxidation, malondialdehyde and medium-weight molecules. Spotted an accumulation of catabolites secondary lipid peroxidation MDA and middle molecules in the sperm that indicates a shift of lipid peroxidation and the development of endogenous intoxication in male germ cells, in men living of the Aral Sea region.

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PP7

Lycopene Inhibits House Dust Mites-Induced TLR4 Activation and Oxidative Stress in Respiratory Epithelial Cells

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House dust mites (HDM) are critical factors causing airway inflammation. HDM are known to activate respiratory epithelial cells to produce pro-inflammatory cytokine (IL-6, IL-8) and trigger toll-like receptor 4 (TLR4) activation. TLR4 mediates ROS production and cytokine expression in some cells. Lycopene is an antioxidant nutrient showing anti-inflammatory activity. In the present study, we investigated whether HDM induce activation of TLR4, production of ROS, and expression of cytokines IL-6 and IL-8 in A549 human respiratory epithelial cells. In addition, we examined the inhibitory effect of lycopene on HDM-induced cytokine expression and ROS production in A549 cells. As a result, HDM induced activation of TLR4, production of intracellular and mitochondrial ROS, and expression of IL-6 and IL-8 in A549 cells. TAK242, a TLR4 inhibitor, suppressed HDM-induced production of intracellular and mitochondrial ROS, and cytokine expression in A549 cells. Lycopene inhibited HDM-induced TLR4 activation, intracellular and mitochondrial ROS production, and cytokine expression in A549 cells. In conclusion, HDM activate TLR4, intracellular and mitochondrial oxidative stress, and inflammatory cytokine expression in respiratory epithelial cells. An antioxidant lycopene may be beneficial for preventing HDM-induced cytokine expression by suppressing TLR4 activation and ROS production in respiratory epithelial cells.

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PP8

Crosstalk of JAK1/STAT3 and WNT/β-catenin in *Helicobacter pylori*-induced hyper-proliferation in gastric epithelial cells: Effect of antioxidant lycopene

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Helicobacter pylori (*H. pylori*) infection, as a risk factor for gastric cancer, is associated with hyper-proliferation and oncogene expression in gastric epithelial cells. Previously, we showed that reactive oxygen species (ROS) mediate activation of JAK/STAT signaling and hyper-proliferation in *H. pylori*–infected gastric epithelial cells. Since JAK signaling is known to mediate Wnt/β-catenin pathway in various cells, we investigated the relation of ROS, JAK/STAT activation, and Wnt/β-catenin pathway for hyper-proliferation in *H. pylori*-infected gastric epithelial AGS cells using an antioxidant lycopene. Lycopene, a bright red carotenoid pigment in fruits and vegetables, has anticancer activity in many types of cancers. As a result, *H. pylori* induced hyper-proliferation in parallel with induction and nuclear translocation of β-catenin. For Wnt/β-catenin signaling, *H. pylori* decreased the level of adenomatous polyposis coli (APC), a component of the destruction complex targeting β-catenin, and induced β-catenin target genes (cyclin-E, c-myc) in AGS cells. *H. pylori* activated GSK3β, but reduced APC and Axin in AGS cells. For JAK/STAT signaling, Jak1 and Stat3 were activated in *H. pylori*-infected AGS cells. Jak1 inhibitor AG490 decreased levels of β-catenin and its target genes (cyclin-E, c-myc) and increased the level of APC in *H. pylori*-infected cells. Lycopene inhibited *H. pylori*-induced ROS production, activation of JAK1/STAT3, reduction of APC/AXIN, and induction of β-catenin and its target genes (cyclin-E, c-myc), and hyper-proliferation in AGS cells. In conclusion, lycopene inhibits *H. pylori*-induced hyper-proliferation by suppressing ROS-mediated activation of JAK/STAT and WNT/β-catenin signaling in gastric epithelial cells.

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PP9

Docosahexaenoic acid inhibits oxidative stress and cytokine expression in pancreatic stellate cells exposed to double-stranded RNA or TNF-alpha

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Activated pancreatic stellate cells (PSC) play a pivotal role in the pathogenesis of pancreatic fibrosis and inflammation. Reactive oxygen species (ROS) levels are increased in the serum of the patients with pancreatitis. ROS mediates expression of inflammatory cytokines in various tissues. Docosahexaenoic acid (DHA), omega 3-polyunsaturated fatty acid, shows anti-inflammatory effects in inflammatory diseases. In the present study, we investigated whether DHA inhibited ROS production and cytokine expression in PSC exposed to double-stranded RNA (Poly (I:C)), as a model of viral infection, or TNF-alpha. PSC were isolated from rats and treated with poly (I:C) or TNF-alpha in the presence or absence of DHA. mRNA expressions of MCP-1 and CX3CL1 were assessed using real-time PCR. DCFDA (2',7' –dichlorofluorescin diacetate), MitoSox red and JC-1(5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazoylcarbocyanine iodide) were used to determine intracellular ROS production, mitochondrial ROS production, and mitochondrial membrane potential (MMP), respectively. As a result, TNF-alpha and Poly (I:C) induced increases in intracellular and mitochondrial ROS as well as expression of MCP-1 and CX3CL1, but reduced MMP in PSC. DHA inhibited intracellular and mitochondrial levels of ROS, disruption of MMP, and cytokine expression in PSC treated with Poly (I:C) or TNF-alpha. In conclusion, supplementation of DHA may be beneficial for preventing pancreatic inflammation/fibrosis by inhibiting cytokine expression in PSC.

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PP10

Protective role of HSP70 against oxidative stress-mediated IL-8 expression in ataxia telangiectasia fibroblasts received glutamine deficiency

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Ataxia telangiectasia (AT) is a neurodegenerative and inherited disease caused by a mutation in the ataxia telangiectasia mutated (*ATM*) gene. *ATM* gene is associated with cell cycle arrest, DNA repair, or apoptosis. Reactive oxygen species (ROS) levels were higher in AT cells lacking *ATM* gene than in normal cells. HSP70 is a molecular chaperone which protects the cells from oxidative stress. Glutamine, a conditionally essential amino acid, is converted to glutamic acid which is a component of glutathione (GSH). Previously, we showed that low GSH level and high levels of ROS and IL-8 in AT cells which was cultured in glutamine deficient medium. In the present study, we investigate the mechanism of ROS production in AT cells received glutamine deficiency in related to HSP 70. As a result, glutamine deficiency increased ROS levels and induced NF-kB activation and IL-8 expression in AT cells. HSP70 levels were decreased by glutamine deficiency. Glutamine deficiency-induced increase in ROS and IL-8 expression are inhibited in AT cells transfected with *HSP70*. In conclusion, HSP70 may protect AT cells from oxidative damage by suppressing ROS production and IL-8 expression.

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PP11

Antioxidant screening in hydrogen peroxide-induced oxidative damage in human somatic and embryonic stem cells

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Oxidative stress, defined as the imbalance between reactive oxygen species production and cellular antioxidant systems, is implicated in a wide range of diseases, and has been extensively studied as a potential target for therapeutic intervention by antioxidants. In order to induce long-term oxidative stress, Hs27 (human fibroblasts) and HUES3 (human embryonic stem cells) were exposed to increasing concentrations of hydrogen peroxide (H_2O_2) by bolus addition every 24 hours during a total of 72 hours. Cell viability, determined by alamarBlue® assay, sharply decreased at 64 μM H_2O_2 in Hs27 and at 128 μM H_2O_2 in HUES3. Following the same long-term exposure experimental design, different antioxidants were tested for cytotoxicity in Hs27 cells at increasing concentrations: glycine (GLY), sodium pyruvate (PYR), N-acetylcysteine (NAC), ascorbic acid (ASC), Trolox (TRO), sodium selenite (SEL) and zinc chloride (ZN). Three non-toxic concentrations were selected for each antioxidant to analyze their protective effect in the presence of increasing concentrations of H_2O_2. GLY, ASC, TRO, SEL and ZN showed no protective effect, while PYR and NAC showed dose-dependent protective effect to up to 16 mM H_2O_2 in the presence of 25 mM PYR and up to 256 μM H_2O_2 in the presence of 2.5mM NAC. Then, PYR and NAC were selected to analyze their cytotoxicity and antioxidant effect in HUES3. Cytotoxicity appeared at lower concentrations in HUES3 than in Hs27. One single concentration was selected for each antioxidant. PYR showed protective effect to up to 512 μM H_2O_2 at 2.5mM, although NAC showed no protective effect in HUES3. Consequently, PYR is a powerful antioxidant against H_2O_2-induced oxidative stress in both somatic and embryonic stem cells, while NAC is protective only for somatic cells, and other well-known antioxidants like ascorbic acid were not able to prevent cytotoxicity.

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PP12

γ-Glutamyl cysteine modulates the inflammatory response *via* protein phosphatases

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Acute pancreatitis (AP) is an acute inflammatory process of the pancreatic gland that may lead to severe systemic complications. Cytokines and oxidative stress play a role in the early pathophysiological events of the disease. Previous studies have shown the antioxidant properties of γ-glutamyl cysteine (γ–GC), a metabolic precursor for the synthesis of glutathione.

C57BL/6 mice were treated with cerulein (7 injections each with 50 μg/kg bw). To evaluate the effects of γ-GC, a group of mice with AP was treated with γ-GC (75 mg/kg bw) administered in two doses at 4 and 7 hours after the first cerulein injection. Plasma lipase activity was measured and histological studies were performed to confirm the induction of AP.

The aim of this work was to assess the role of γ-GC in the modulation of the inflammatory response and oxidative stress in AP.

The activity of pancreatic lipase in plasma increased after induction of acute pancreatitis, but this increase was lower with γ-GC treatment. The histopathological study showed that the inflammatory process and tissue edema were reduced with the γ-GC treatment. The increase in pancreatic myeloperoxydase activity and TNF-α mRNA levels were abrogated in mice treated with γ-GC after AP induction. Moreover, the c-Jun N-terminal kinase (JNK) pathway activation was blocked upon γ-GC treatment. On the contrary, protein levels of protein tyrosine phosphatases SHP-1, SHP-2 and protein serine threonine phosphatase PP2A, which were reduced upon AP induction, were recovered after γ-GC administration. Redox pairs, such as reduced glutathione/oxidized glutathione, and cysteine/cystine were not affected by the γ-GC treatment.

In conclusion, our results show the anti-inflammatory properties of the γ-GC in AP, which seem to be mediated by recovering protein phosphatase levels and avoiding the activation of the JNK pathway, independently of the redox thiol status.

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PP13

The protein kinase 2 inhibitor tetrabromobenzotriazole protects against renal ischemia reperfusion injury

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Protein kinase 2 (CK2), activated by growth factor receptors, has a profound influence on cell proliferation and survival. Recently, CK2 activation was reported to enhance reactive oxygen species production and activate the nuclear factor κB (NF-κB) pathway. Because oxidative stress and inflammation are critical events for tissue destruction during ischemia reperfusion (I/R), we sought to determine whether CK2 was important in the renal response to I/R. Mice underwent 25 min of renal ischemia and were then reperfused. We confirmed an increased expression of CK2α during the reperfusion period, while expression of CK2β remained consistent. We administered tetrabromobenzotriazole (TBBt), a selective CK2α inhibitor before inducing I/R injury. Mice subjected to I/R injury showed typical patterns of acute kidney injury; blood urea nitrogen and serum creatinine levels, tubular necrosis and apoptosis, inflammatory cell infiltration and proinflammatory cytokine production, and oxidative stress were markedly increased when compared to sham mice. However, pretreatment with TBBt abolished these changes and improved renal function and architecture. Suppression of nuclear factor-κB (NF-κB) and mitogen activated protein kinase (MAPK) pathways might have caused the renoprotective effects of TBBt. Taken together, these results suggest that CK2α mediates proapoptotic and proinflammatory signaling, thus the CK2α inhibitor may be used to prevent renal I/R injuries observed in clinical settings.

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PP14

Aggravation of post-ischemic liver injury by over-expression of insulin-like growth factor binding protein 3

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Insulin-like growth factor-1 (IGF-1) is known to inhibit reperfusion-induced apoptosis, thus preventing hepatic ischemia/reperfusion (I/R) injury. IGF-binding protein-3 (IGFBP-3) is the major circulating carrier protein for IGF-1 and induces apoptosis. In this study, we determined if IGFBP-3 was important in the hepatic response to I/R. To deliver IGFBP-3, we used an adenovirus containing IGFBP-3 cDNA (AdIGFBP-3) or an IGFBP-3 mutant devoid of IGF binding affinity but retaining IGFBP-3 receptor binding ability (AdIGFBP-3^{GGG}). Mice subjected to I/R injury showed typical patterns of hepatocellular damage. Protein levels of IGFBP-3 were increased after reperfusion and showed a positive correlation with the extent of liver injury. Prior injection with AdIGFBP-3 aggravated liver injury: serum aminotransferases, prothrombin time, proinflammatory cytokines, hepatocellular necrosis and apoptosis, and neutrophil infiltration were markedly increased compared to control mice. A decrease in antioxidant potential and an upregulation of NADPH oxidase might have caused these aggravating effects of IGFBP-3. Experiments using HepG2 cells and N-acetylcysteine-pretreated mice showed a discernible effect of IGFBP-3 on reactive oxygen species generation. Lastly, AdIGFBP-3 abolished the beneficial effects of ischemic preconditioning and hypothermia. Mice treated with AdIGFBP-3^{GGG} exhibited effects similar to those of AdIGFBP3, suggesting a ligand-independent effect of IGFBP-3. Our results suggest IGFBP-3 as an aggravating factor during hepatic I/R injury.

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PP15

Interplay between lipid and protein carbonylation in a cardiomyocyte model of nitrosative stress

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Carbonylation of biomolecules, a stable oxidative modification yielding reactive carbonyl groups, is a well-accepted biomarker of oxidative damage, which has been linked to several oxidative stress (OS) related human diseases. Protein carbonylation can be induced via several independent mechanisms, including direct oxidation of amino acid residues or modification of

nucleophilic residues with products of lipid and carbohydrate oxidation. Lipid-protein adducts are believed to be a major source of protein-bound carbonyls. Lipid peroxidation products (LPP) containing reactive carbonyls can modify proteins in two different ways via - (i) Schiff base formation with a loss of the carbonyl function or (ii) Michael addition resulting in protein carbonylation. Although oxidative modifications of lipids and proteins in biological systems are closely interconnected, they are rarely studied together. Here we combined a mass spectrometry (MS) based analysis of OS-derived LPP with proteomics targeting modified proteins for high-throughput identification of lipid-protein adducts in a dynamic cellular model of OS.

The influence of nitrosative stress on carbonylation of proteins and lipids was investigated in primary cardiomyocytes treated with SIN-1 for different time intervals. Twenty-five carbonylated lipids were identified by LC-MS and considered as possibly modifying agents yielding the corresponding LPP-modified proteins. A combination of different proteomics techniques allowed identifying over 200 of these modified proteins. Systems biology analysis of modified proteins revealed alterations in Ca^{2+} -signalling pathways. Ca^{2+} mobilization was studied by activating voltage-dependent Ca^{2+} channels, ryanodine receptor 2 and inositol-trisphosphate receptor. Significant alterations in the activity of LPP-modified Ca^{2+} -channels were observed after 70 min of SIN-1 treatment. Thus, the combination of lipidomics, proteomics, and biochemical assays allowed connecting the molecular pattern of "carbonylation stress" to functional changes in the studied nitrosative stress cell model.

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PP16

Fluorescent microscopy study of intracellular distribution of carbonylated proteins and lipids

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Carbonylation is an important oxidative modification of biomolecules and widely accepted as a biomarker of oxidative stress (OS). Carbonylated proteins, lipids, and nucleic acids have been intensively studied and were often linked to the onset or progression of OS related disorders. Carbonylated species are usually identified and quantified in cell lysates and body fluids after derivatization with specific chemical probes. Several analytical methods for identification and quantification of carbonylated biomolecules have been reported. However, in order to understand cellular carbonylation pathways and reveal their biological relevance it is crucial to study their intracellular formation and spatial distribution. Here, we used coumarin-hydrazide, a fluorescent chemical probe, for time- and cost-efficient labeling of cellular carbonyls followed by fluorescence microscopy to evaluate their intracellular formation both in time and space.

The protocol was verified in a cellular model of paraquat induced OS and compared with a conventional DNPH-based immunocytochemistry. The specificity of coumarin-hydrazide towards carbonylated proteins and lipids was confirmed by a wide range of analytical techniques including gel electrophoresis, thin layer chromatography, and mass spectrometry. Additionally, co-distribution with oxidized lipids was evaluated by confocal microscopy using oxidized phosphatidylcholine specific natural antibodies. We have applied this method to detect carbonyls in several cellular models of oxidative stress including paracetamol induced hepatocyte toxicity and nitrosative stress in primary cardiomyocytes. A strong increase in biomolecule oxidation was observed in all studied OS models and most of the carbonylated species were accumulated in perinuclear space. Using confocal microscopy, co-localization of carbonylated biomolecules with endoplasmic reticulum was also demonstrated.

Keywords: Coumarin-hydrazide; Dinitrophenyl hydrazine; Fluorescence microscopy; Protein and lipid carbonylation; Spatial distribution

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PP17

Kinetic investigation of the reaction of 4-methylbenzoquinone with thiol and amine compounds: consequences for protein modification

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Plant extracts rich in polyphenols are added as antioxidants to various foods. Oxidation of polyphenols to quinones serves as an antioxidative mechanism, but the resulting quinones may induce damage to proteins as they react through a Michael addition with nucleophilic groups, such as thiols and amines to give protein adducts. The reaction of quinones with amino acids is involved in many physiological and pathological phenomena, such as skin inflammation, cataract and bone marrow toxicity. In this project we have determined rate constants for reaction of 4-methylbenzoquinone (4MBQ) (the oxidized form of 4-methylcatechol; generated by electrolysis) with thiols and amines (L-cysteine, N-α-acetyl-L-cysteine, glutathione (GSH), L-glycine, N-α-acetyl-L-lysine, N-α-acetyl-L-arginine) under pseudo first-order conditions. The rate constants were determined by following the loss of 4MBQ at 401 nm, and adduct formation at 294 nm by stopped-flow spectrophotometry over the pH range 4.5-6.5.

Autoreduction of 4MBQ has $k \sim 0.073\ s^{-1}$ at pH 6.5. With L-glycine k is $0.102\ s^{-1}$ at pH 6.5 so this is a slow reaction. No reaction was observed with side chain amine groups. Reaction of thiols with quinones occurs via reversible formation of an intermediate (with equilibrium constant K) and subsequent irreversible conversion to the final phenol adduct, with rate constant, k. With the thiols examined, the highest rate constant was obtained for L-cysteine (k 2150 s^{-1}). Lower values were obtained for N-α-acetyl-L-cysteine, where the amino group of is blocked by acetylation, and for GSH, suggesting that a free amine increases the reaction rate. These data shows that thiol groups are major targets for quinones such as 4MBQ, with rate constants at least 10,000-fold greater than for reaction with the side chain amine group of Lys, the free amino acid of amino acids, and the guanidine group of Arg residues. Thiols are therefore the major kinetic targets for protein modifications by quinones.

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PP18

Optimisation of chromatographic separation of oxidised phospholipids and detection with targeted approaches provides greater coverage of the oxidised lipidome

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Phospholipid oxidation by adventitious damage generates a wide variety of products with potentially novel biological activities that can modulate inflammatory processes associated with various diseases. To understand the biological importance of oxidised phospholipids (OxPL) and their potential role as disease biomarkers requires precise information about the abundance of these compounds in cells and tissues. There are many chemiluminescence and spectrophotometric assays available for detecting oxidised phospholipids, but they all have some limitations. Mass spectrometry coupled with liquid chromatography is a powerful and sensitive approach that can provide detailed information about the oxidative lipidome, but challenges still remain.

The aim of this work is to develop improved methods for detection of OxPLs by optimisation of chromatographic separation through testing several reverse phase columns and solvent systems, and using targeted mass spectrometry approaches. Initial experiments were carried out using oxidation products generated in vitro to optimise the chromatography separation parameters and mass spectrometry parameters. We have evaluated the chromatographic separation of oxidised phosphatidylcholines (OxPCs) and oxidised phosphatidylethanolamines (OXPEs) using C8, C18 and C30 reverse phase, polystyrene – divinylbenzene based monolithic and mixed – mode hydrophilic interaction (HILIC) columns, interfaced with mass spectrometry. Our results suggest that the monolithic column was best able to separate short chain OxPCs and OxPEs from long chain oxidised and native PCs and PEs. However, variation in charge of polar head groups and extreme diversity of oxidised species make analysis of several classes of OxPLs within one analytical run impractical. We evaluated and optimised the chromatographic separation of OxPLs by serially coupling two columns: HILIC and monolith column that provided us the larger coverage of OxPL species in a single analytical run.

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PP19

Diet affects the redox system in developing Atlantic cod (*Gadus morhua*) larvae

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The growth and development of marine fish larvae fed natural copepods is superior to those fed rotifers, but the underlying molecular mechanisms are unclear. In this study we compared the effects of such diets on redox regulation pathways during development of Atlantic cod (*Gadus morhua*) larvae. Cod larvae were fed a control diet of copepods comprising species that are typical feed for cod in nature, or the standard rotifer/*Artemia* diet commonly used in commercial marine fish hatcheries, from first feeding until after metamorphosis. The oxidized and reduced glutathione levels, the reduction potential, and the mRNA expression of 100 genes in the redox system pathways were compared between the two treatments and at different defined stages during larval development. Growth was similar in the two groups the first 22 days after hatching, thereafter the growth was 2-fold higher in the copepod-fed fish until both groups were weaned onto a formulated diet. We found that rotifer/*Artemia*-fed cod larvae had generally lower levels of oxidized glutathione, a more reduced redox potential, and altered expression of nearly half of the redox system genes when compared to copepod-fed larvae. The rotifer/*Artemia* diet-induced an apparent dysregulation of the redox system that was greatest during periods of suboptimal growth. Upregulation of the oxidative stress response transcription factor, *nfr2*, and NFR2 target genes in rotifer/*Artemia* fed larvae suggest this diet induced an NFR2-mediated oxidative stress response. Overall, the data demonstrates that nutrition plays a major role in the redox regulation of developing fish larvae. This may well be a key factor for the dietary-induced differences observed in larval growth. The nutrient composition of diets and larvae were analysed and we will present an evaluation of how the different nutrients may have contributed to differences in growth and redox biology.

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PP20

Plasma carotenoids, tocopherols, and retinol: Associations with age and demographic characteristics in the age-stratified general population of the European MARK-AGE study

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Dietary patterns as well as plasma concentrations of carotenoids, tocopherols, and retinol may differ between age groups and therefore may be associated with aging.

For the MARK-AGE Project, a European multicentre study aimed to identify biomarkers of human aging, randomly recruited women and men from an age-stratified general population (35 - 75 years), as well as subjects from long-living families were recruited. Within this study we analyzed plasma micronutrients (six carotenoids, α- and γ-tocopherol, and retinol) among 2,118 participants.

Plasma lycopene and α-/β-carotene were inversely correlated with age whereas β-cryptoxanthin, lutein, zeaxanthin, α-/γ-tocopherol and retinol were positively associated. The strong inverse association of lycopene and α-carotene with age and the positive association of α-tocopherol and β-cryptoxanthin with age were confirmed in multiple- and cholesterol-adjusted regression models. Age, country, season, and cholesterol were the main predictors for plasma concentrations of lycopene, α-tocopherol, β-cryptoxanthin, and α-carotene and were significantly affected by gender,

smoking status, BMI, and dietary pattern.

The decrease of lycopene with age remained after adjustment for significant co-factors and covariates, whereas the increase in α-tocopherol with age was less pronounced if all covariates including cholesterol and servings of vitamin supplements were assessed.

The results of the MARK-AGE study suggest age as an independent predictor of plasma lycopene, α-tocopherol, and α-carotene. These micronutrients, together with other markers, may contribute to a set of biomarkers of human aging.

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PP21

The degradation of Vitamin C by reactive oxygen species

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Vitamin C (ascorbate and dehydroascorbic acid) is vital for plants and found throughout the plant cell including in the apoplast. The structure of ascorbate was determined eighty years ago; however, many of its degradation pathways remain unclear. Numerous degradation products of ascorbate have been reported to occur in the apoplast but many still remain unidentified[1,2]. Ascorbate is well known as an antioxidant, and acts to quench reactive oxygen species (ROS), such as hydrogen peroxide and ozone in the plant apoplast. The immediate oxidation product of ascorbate is dehydroascorbic acid (DHA), which may be quickly hydrolysed to diketogulonic acid (DKG). The further reactions of radiolabelled and non-radiolabelled DHA and DKG with various ROS have been investigated. The resulting oxidation products were separated by high-voltage paper electrophoresis. Differences were observed in the products formed from the various ROS, allowing a unique fingerprint of oxidation products to be described for each ROS. Equally, different compounds were produced depending on the starting substrate; for example cyclic oxalyl threonate was only observed in the reactions of DHA and not DKG.

Vitamin C is also a vital component of the human diet, and most dietary ascorbate comes from plants such as salads. The degradation of ascorbate during post-harvest processing and storage of salad leaves has been investigated. Studies are being conducted to determine the presence of ascorbate oxidation products within salad leaves.

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PP22

Liver antioxidant defense after FAAH inhibitor - URB-597 administration to DOCA-salt-induced hypertension in rats

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Oxidative stress is involved in progression of hypertension therefore the influence on endocannabinoids level may be useful in pharmacotherapy. The increase in the main endocannabinoid - anandamide level may be attained by inhibition of FAAH –enzyme responsible for anandamide degradation. Therefore the aim of this study was to exam complex dependencies between endocannabinoid system and oxidative stress formation as well as consequences of observed changes in the liver of hypertensive rats [DOCA-salt] receiving URB597 - FAAH inhibitor. 4-5 weeks old rats after nephrectomy have been receiving DOCA twice weekly and replacement of drinking water with 1% NaCl solution for 6 weeks. One group of these rats was injected i.p. with URB597 (1 mg/kg b.w.) for last 14 days. It has been shown that URB597 given to hypertensive rats caused an increase in the level of AEA and expression of CB1, CB2 receptor as well as decrease in the level of 2-AG and the activity of FAAH and MAGL what was accompanied by an increase in free arachidonic acid. URB597 caused also the significant changes in liver redox status. The ROS, GSH, GSSG, vitamin A levels and activity Cu,Zn-SOD, CAT, GSH-T were increased, while the activity of GSH-Px, GSSG-R as well as the level of vitamin C, E were diminished in comparison to hypertensive rats. The antioxidant defense was changes also on transcriptional level. Results showed that URB597 administration enhanced level of Nrf2 and its activator - cJun and caused decrease in other Nrf2 activators [KAP1, p21, p62, ERK1/2, pERK1/2] and its inhibitors [Keap1, Bach1]. Moreover expression of HO-1 was decreased. The consequence of above disturbances in redox status was increase in oxidative damages of lipid (4-HNE, MDA, 8-isoprostanes), protein (dityrosine, tryptophane, carbonyl group) and DNA (8-OH dG).

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PP23

Tick-borne encephalitis - lipid peroxidation and its consequences

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The purpose of this study was to assess the processes of lipid peroxidation with prostaglandin derivatives and reactive aldehydes being its major indicators in cerebrospinal fluid (CSF), plasma and urine of patients with

tick-borne encephalitis (TBE). This study included 60 patients with TBE and 56 healthy subjects. Lipid peroxidation was estimated by the measurement of 4-hydroxynonenal (4-HNE), 4-hydroxyhexenal (4-HHE), malondialdehyde (MDA), acrolein, crotonaldehyde, and 4-oxononenal (4-ONE), determined by GC–MS, F2-isoprostanes and neuroprostanes (NPs) level determined by LC-MS. The level of 4-HNE-protein adducts was determined by ELISA. Phospholipase A2 (PLA2), platelet-activating factor acetylhydrolase (PAF-AH) and glutathione peroxidase (GSH-Px) activities and vitamin E level were determined spectrophotometrically and by HPLC, respectively. In parallel, the plasma levels of phospholipid acids such as arachidonic acid (AA), linoleic acid (LA) and docosahexaenoic acid (DHA) were monitored. Results. Significant decrease in AA, LA, DHA level and GSHPx activity (by about 20%, 69%, 11% and 18%, respectively) was observed. The consequence of enhanced phospholipid peroxidation was almost 7-times higher plasma level of F2- isoprostanes and 3-fold increase in NPs level in CSF of TBE patients. Additionally 3.5-fold increase in the CSF level of MDA, 5-fold increase in the plasma level of 4-HNE and urine level of 4-HHE in TBE patients was observed. Decreased plasma activity of PLA2 with an increase in the PAF-AH activity was observed. Lipid peroxidation occurring during TBE development indicates its relevance in pathophysiology of this disease. Moreover lipid peroxidation products might be useful for the diagnosis of TBE.

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PP24

Heat shock proteins in proteasome inhibitor related neuropathy

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The proteasomal system seems to be a promising target in the cancer therapy due to the different levels and activities of its components in cancer and normal cells. Synthetic and naturally occurring proteasome inhibitors are used in research and also in clinical studies. Bortezomib (N-pyrazinecarbonyl-L-phenylalanine-L-leucine boronic acid) first-in-class proteasome inhibitor was approved by the FDA for the treatment of mantle cell lymphoma in 2006 and of multiple myeloma in 2008. Peripheral neuropathy is a common and often dose-limiting toxic side effect of bortezomib as many other active chemotherapeutic agents. Since bortezomib is the mostly used proteasome inhibitor in the clinic, several studies have been carried out to highlight the underlying mechanisms of Bortezomib Induced Peripheral Neuropathy (BINP) but current knowledge is still scarce and contradictory.

Our first study was focused on identifying the possible proteins involved in the bortezomib induced neuropathy and also possible differences between bortezomib- and carfilzomib-treated cell proteomes because carfilzomib is believed to be less neurotoxic compared to bortezomib. In this direction, we treated neural stem cells with bortezomib and carfilzomib. Proteins were extracted from bortezomib and carfilzomib treated neural cells as well as from untreated cells using 1% β-octyl glucopyranoside lysis buffer. Extracts were tryptically digested on 3 kDa spin filters and the peptides were analyzed by nanoLC-MS/MS using a 7 T hybrid LTQ-FT mass spectrometer. Protein identification was carried out using MASCOT search engine with a confidence level of 95% and proteins were quantified using MaxQuant software. According to the results of the study, we showed significant changes for Heat Shock Proteins (HSPs) in different study groups besides many other proteins involved. Since HSPs have crucial roles in protein homeostasis, the possible involvement of these proteins in BINP has been discussed.

Keywords: Proteasome inhibitors; bortezomib; carfilzomib; neuropathy; heat shock proteins

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PP25

Inflammation-related DNA damage in relation to the expression of cancer stemness markers in human nasopharyngeal carcinoma

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Reactive nitrogen species are considered to participate in inflammation-related carcinogenesis through DNA damage. 8-Nitroguanine is a specific marker of inflammation-related carcinogenesis. Epstein-Barr virus infection-related nasopharyngeal carcinoma (NPC) is one of the most prevalent malignant tumors in southern China and Southeast Asia, and its prognosis has been poor for decades. Previously, we demonstrated that nitrative DNA damage, such as 8-nitroguanine formation, occurs in cancer cells of NPC patients (Ma et al. Int. J. Cancer 2008). In the present study, to investigate the involvement of stem cells in NPC, we performed immunohistochemical analyses to examine nitrative DNA lesions (8-nitroguanine) and several cancer stem / progenitor cell markers (CD44v6, CD24 and ALDH1A1) in nasopharyngeal tissues obtained from chronic nasopharyngitis and NPC patients. The staining intensity of 8-nitroguanine was significantly higher in cancer cells and inflammatory cells in stroma of NPC than in chronic nasopharyngitis tissues. Expression levels of CD44v6 and ALDH1A1 were significantly increased in cancer cells of primary NPC specimens in comparison to chronic nasopharyngitis tissues. 8-Nitroguanine was detected in CD44v6- or ALDH1A1-positive stem cells in NPC tissues. In the case of CD24 staining, there was no significant difference between NPC and chronic nasopharyngitis tissues. Intensive staining of CD44v6 and ALDH1A1 were also detected in NPC cell line HK1 compared to immortalized nasopharyngeal epithelial cells NP460 by a double immunofluorescence study and western blotting assay. In conclusion, CD44v6 and ALDH1A1 could be candidates of cancer stem cell markers for NPC. The present results indicate the possible mechanism by which inflammation causes NPC by inducing inflammatory processes and formation of 8-nitroguanine in CD44v6- and / or ALDH1A1-positive stem cells, and mutant stem cells participate in NPC development.

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PP26

Liver fibrosis is improved by treatment of S-allyl cysteine, an aged-garlic extract, in CCl_4-treated rats

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Objectives: S-allyl cysteine (SAC) is the most abundant compound in aged garlic extracts (AGEs). AGE has been reported to ameliorate the oxidative damage implicated in a variety of diseases. However, the effects of SAC have not been established in liver cirrhosis. The aim of this study was to examine the survival and the effect of therapeutic administration of SAC in the established liver fibrosis by chronic carbon tetrachloride (CCl_4) administration in rats.

Materials & Methods: Male Wistar rats (4 weeks age) were used. Cirrhosis was induced by chronic CCl_4 (2 ml/kg, 25% in corn oil, twice a week) intraperitoneal administration. Four weeks after CCl_4 treatment, SAC or cysteine compounds (cysteine, N-acethyl cysteine) were given by mixed diets for 8 weeks (CCl_4:total 12 weeks) or longer (survival rate).

Results: CCl_4 administration elevated plasma alanine aminotransferase, plasma lipid peroxidation, liver hydroxyproline, and liver transforming growth factor (TGF)-beta at 12 weeks. SAC markedly normalized these changes induced by CCl_4 than other cysteine compounds. Furthermore, SAC improved survival in a dose-dependent manner following consecutive CCl_4 administration. The inhibitory mechanisms may be associated with a decrease in the profibrogenic cytokine, TGF-beta as well as the antioxidative properties of SAC.

Conclusion: Therapeutic administration of SAC improved liver fibrosis developed by CCl_4 and survival rate. These findings suggest SAC is a beneficial candidate for therapeutic agents on the liver cirrhosis.

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PP27

The cross-talk between oxidative stress and endocannabinoid system in keratinocytes after UV irradiation

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Daily skin exposure to UV radiation may cause skin damage connected mainly with imbalance in the redox status and metabolic disturbances. Therefore to evaluate the cross-talk between oxidative stress and endocannabinoid system of skin cells following UV, keratinocytes were subjected to UVA and UVB irradiation [30 J/cm^2 and 60 mJ/cm^2, respectively]. The redox status was estimated by ROS generation and oxidative modifications of lipid and protein were examined. The endocannabinoids and their receptors levels were also measured.

Our results showed that UV irradiation caused an increase both in ROS generation and in lipid peroxidation markers in tested cell line. UVA irradiation resulting in even a two-fold increase in the level of 4-HNE in keratinocytes, whereas UVB exerted the strongest increase in 4-HHE level. UVA caused a stronger increase in MDA level than UVB radiation. Simultaneously an increase level of protein oxidative modifications markers such as dityrosine and carbonyl groups was observed. Obtained results also demonstrated that UV irradiation affected the endocannabinoid system. In the case of UVA irradiation, applied dose leads to 2 times reduction in the AEA level in compared to control cells, while UVB irradiation reduces it about 5 times. Further, it was observed that the exposure of cells to UVB irradiation significantly reduced the level of 2-AG. Parallel to these changes a reduction in the amount of endocannabinoids receptors CB1, CB2 and TVR1 in cytoplasm and their increased activation in membrane fraction after cells exposure to UVA or UVB irradiation was shown. In addition, there was a higher expression of Nrf2 and all Nrf2 activators induced stronger by UVB than UVA radiation.

Obtained results demonstrate a strong association between changes in endocannabinoid signalization and UV-induced oxidative stress in keratinocytes, which may be important point to searching for the skin protecting method from harmful ultraviolet radiation.

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PP28

Inhibition of Myeloperoxidase-Mediated Endothelial Dysfunction by Nitroxides

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The leukocyte-derived heme enzyme myeloperoxidase (MPO) has emerged as a key therapeutic target in vascular inflammatory diseases, where it is implicated as a mediator of endothelial dysfunction. During inflammation, MPO is released by activated leukocytes and accumulates subcellularly within the endothelium. Here, it mediates endothelial dysfunction by catalytically consuming nitric oxide (NO) and producing reactive oxidants (e.g. hypochlorous acid (HOCl) and $NO_2^{\bullet}$) that damage extracellular matrix proteins, impair vascular NO biosynthesis, and stimulate vascular superoxide production.

Tempol and related nitroxides have been shown to exert protection in a variety of inflammatory conditions. Although this is widely thought to reflect their actions as superoxide dismutase mimetics; we and others have shown that nitroxides can also effectively inhibit MPO-catalysed HOCl production and protein nitration in simple model systems (Biochem. J., 2009;421:79-86; PNAS, 2008;105:8191-6). However the capacity of nitroxides to protect the endothelium against MPO-mediated damage, as well as structural features that may optimise this activity, have yet to be examined.

Here, we synthesised a range of novel nitroxides bearing endothelial-targeting moieties in order to optimise delivery to endothelial subcellular compartments where MPO and its reactive products localise. Both simple and novel nitroxides were screened for their ability to inhibit endothelial-localised MPO chlorination and nitration reactions in cultured endothelial cells and in *ex vivo* rat aorta, as well as MPO-catalysed NO consumption in human plasma.

Our data identified for the first time that nitroxides exert vascular protection via MPO inhibition as well as radical scavenging. Furthermore, our data indicated that vascular targeting of nitroxides to endothelial subcellular compartments can enhance protection, particularly against

radical-mediated reactions (i.e. protein nitration and MPO-catalysed NO consumption). Overall, nitroxides are ideal for development as MPO-targeted therapeutics.

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PP29

Regulation of Myeloperoxidase NO Oxidase Activity by Pharmacological Agents: Novel Biochemical Basis for NO Preservation by Phenolics and Nitroxides (Tempol)

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Introduction: The leukocyte-derived heme enzyme myeloperoxidase (MPO) plays a key role in impairing NO bioavailability during inflammatory conditions. MPO can consume NO as a direct enzyme substrate as well as by generating NO-consuming free radicals. While our recent studies reveal that endogenous agents (thiocyanate, ascorbate) inhibit this NO oxidase activity, their protective action is incomplete (*FRBM,* 2014;72:91-103).

Aims: We sought to identify pharmacological agents that can afford additional protection against MPO NO oxidase activity in complex physiological fluids. We also sought to identify drugs that stimulate this activity, as these may have unforeseen side-effects.

Methods: Diverse pharmacological agents with established redox activities as MPO substrates and/or radical scavengers were screened for their effects on MPO NO oxidase activity in human plasma and physiological model systems containing endogenous MPO substrates and antioxidants.

Results: Unexpectedly, the hydrazide MPO inhibitors ABAH and isoniazid, and the peroxidase-activated NO donor hydroxyurea all greatly stimulated MPO NO oxidase activity under physiological conditions. Melatonin marginally increased NO consumption. The phenolic drugs acetaminophen and resveratrol, which are excellent peroxidase substrates, initially stimulated NO consumption but ultimately limited the extent of NO losses. Tempol and related nitroxides were uniquely able to inhibit the rate of MPO-catalyzed NO consumption in ascorbate-replete fluids.

Discussion: Kinetic analyses revealed the mechanistic basis of drug activities and identified criteria for developing drugs with improved activity. Overall, our data reveal that widely-used pharmacological agents strongly influence MPO NO oxidase activity in complex physiological fluids and identify novel mechanisms by which phenolic drugs and nitroxides may preserve NO bioavailability during inflammatory conditions.

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PP30

Assessment of the histone like proteins treatment in men's sperm, living at Aral Sea Region

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The priority task of governmental politic in modern conditions is the scientific treatment of the environmental factors influence on the population health in environmentally unfavorable regions. In Aral area was formed difficult complex, consisted of environmental and demographic problems that led to deterioration of the environment. Numerous studies conducted by the researchers of Kazakhstan proved that the state of health of the population continues to deteriorate Aral Sea region.

The big problem on the environmental stability is providing - dust salty – aerosol, heavy metals, and nitrates. Literature data are proving negative environmental factors influence on genetic stability, situation with the immune system and endocrine system, pathologies.

At the same time, there is less studied the condition of reproductive health in men population, living at the regions of environmental disaster and crisis of Aral area, cause in modern conditions there is an actual study of pathological states under the influence of negative factors.

Aim of the research: to study contamination change of the histone like proteins in men sperm, living at Aral Sea regions.

Materials and methods: The object of study was the sperm of men, living at the zone of environmental disaster - city Aralsk and zone of environmental crisis – village Zhalagash, that were divided on 3 age groups: 1-st group - 18-29 years, 2-nd group - 30-39 years, 3-rd group – 40 - 49 years.

Results and discussions: A significant increase of histone like proteins in sperm of men of the 3 age groups living in the zone of ecological disaster of Aral city in comparison with groups of men living in the village Zhalagash and the control values.

Conclusion: The results of our study can serve as an indirect indicator of the pathological process development in spermatozoa and activation of the genetic apparatus under the influence of negative environmental factors.

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PP31

Changing of extracellular nucleic acids and histone like proteins in blood of patients with complications of peptic ulcer disease

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Peptic ulcer disease - a chronic relapsing poly etiologic disease is widespread throughout the world. Among the patients who are hospitalized with gastrointestinal diseases, peptic ulcer disease is diagnosed in 35.8% of cases. Implementation of peptic ulcer disease is carried out by the interaction of exogenous and endogenous factors: genetic predisposition, such as the nervous and endocrine systems, psycho-emotional characteristics, the characteristics of metabolic, biochemical reactions. In modern conditions, there is a noticeable increase in the incidence of peptic ulcer caused by stressful situations, bad habits, use of drugs, resulting in impaired balance between "aggressive" factors and "protective" factors of gastroduodenal zone.

Aim of research: Investigating of extracellular nucleic acid contamination changing and histone like proteins in blood of patients with complications of peptic ulcer disease.

Material and methods: The object of the study was the blood of patients with ulcer complications, which were determined precursors of nucleic acids and histone like proteins.

Results of study: A significant increase in the fraction 1.9 times H1, H2A, and H3, H4 fraction in 3.5 times in comparison with the control values and a significant decrease H2B fraction 1.5. The quantity of ASF in the blood of patients with peptic ulcer disease significantly increased by 1.2 times compared with the control, with corresponding reduction of circulating extracellular nucleic acids.

Conclusion: Changing the content of histone like proteins extracellular nucleic acids, in patients with a complication of gastric ulcer, can be explained by "breakdown" compensatory mechanisms adapted by the action of unfavorable factors that lead to oxidative degradation of histone proteins and metabolic disorders predecessors nucleic acids.

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PP32

Hsp70 promotes the proteolysis of oxidatively damaged proteins

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Due to its function as molecular chaperone, Hsp70 is involved in various mechanisms maintaining the proteome integrity. Next to its ability to refold misfolded or damaged proteins it has been shown that Hsp70 and other members of the heat shock protein family (HSPs) are involved in proteolytic processes. HSPs are involved in the stabilization of components of the proteasomal system and may be even directly involved in degradation processes. There is evidence that Hsp70, together with its co-chaperones, is able to recognize substrate proteins for the 26S proteasome. In addition, a direct interaction of Hsp70 with the 26S proteasome has been demonstrated. However, until now there is no evidence indicating a direct involvement of Hsp70 in proteasomal degradation of oxidized proteins via the 20S proteasome.

We have previously demonstrated the involvement of Hsp70 in proteasomal degradation of oxidized proteins. On the one hand, Hsp70 knockdown leads to an accumulation of oxidized proteins without influencing the proteasomal activity. On the other hand, *in vitro* experiments demonstrated that the presence of Hsp70 increases the degradation of oxidized proteins via the 20S proteasome. However, the underlying mechanism of Hsp70 in promoting the degradation of oxidatively damaged proteins must to be clarified. We have already shown an increased interaction of Hsp70 with oxidized proteins after oxidative stress. To prove the hypothesis that Hsp70 may be involved in recognition and transport of these oxidized proteins to the 20S proteasome, in further experiments we intend to investigate whether there is also an enhanced interaction of Hsp70 with the 20S proteasome in the recovery phase after oxidative stress.

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PP33

Impact of tyrosine nitration on cellular glutamine synthetase turnover and functionality

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During oxidative damage to proteins by reactive nitrogen species (RNS), post-translational modifications can lead to dysfunctional proteins and promote their aggregation over time. One of the protein modifications *via* RNS, such as peroxynitrite ($ONOO^-$), is protein tyrosine nitration. It is based on an initial one-electron oxidation of the aromatic phenol ring of tyrosine followed by the addition of $ONOO^-$-derived products, such as nitric dioxide. For glutamine synthetase (GS), an important enzyme for the detoxification of ammonia in brain, it has been shown that $ONOO^-$ is able to impair enzyme activity. Thus, we are investigating the link between protein nitration and the loss of enzyme function, to clarify under which conditions the protein activity is declining. Therefore, cellular GS from human astrocytes as well as the human recombinant protein (rGS) were studied. Nitration was induced by the $ONOO^-$ donor SIN-1 and quantified immunochemically by an anti-protein-3-Nitrotyrosine (3-NT) antibody. Besides the studies on the formation of nitrated GS and its nitration state after a recovery time, we further followed the impact of SIN-1 on enzyme functionality by a GS activity assay. In order to follow the degradation of GS, we added RAW 264.7 cell lysates (w and w/o LPS) to nitrated GS and analyzed it on protein and activity level. Preliminary results showed an increased formation of $ONOO^-$ and an induction of 3-NT after incubation with SIN-1. Further, GS activity was decreased by SIN-1 in both, cellular GS and rGS. After a 3h-recovery period, GS activity increased again in cells. Removing of 3-NT-modified proteins by a putative denitration system might be a possibility to maintain protein function and impede protein aggregation.

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PP34

The Nrf2-ARE activation protect neurones against oxidative stress

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Oxidative stress has been implicated in the pathogenesis of many neurodegenerative diseases including Alzheimer's disease. The transcription factor, Nrf2 (nuclear factor E2-related factor 2) that binds to the antioxidant responsive element (ARE) activates a battery of genes encoding enzymes and factors essential for neuronal survival. We have investigated the hypothesis that a downstream product of cyclooxygenase (COX-2), 15-deoxy-delta (12, 14)-prostaglandin J2 (15d-PGJ2) has protective effects by activating the Nrf2 pathway during oxidative stress.

Human neuroblastoma cells (SHSY5Y) were differentiated into neuronal-like cells as described previously (Gimenez-Cassina et al., 2006). SHSY5Y cells were co-treated with 10 μM buthionine sulfoximine (BSO) ± 10 μM 15d-PGJ2. Cell viability was measured by MTT assay and cellular glutathione (GSH) levels were measured after treating cells for 0.5-24 hours by GSH recycling assay. Cellular Nrf2 levels were determined by immunoblotting. IL-6 levels were measured by ELISA.

15d-PGJ2 alone lowered GSH levels 30min after the treatment (12.8 ± 0.64 nmol/mg protein) and returned to untreated control levels at 16hours (28.1 ± 3.6 nmol/mg protein; $P < 0.01$). Compared to intracellular GSH levels in untreated cells (27.8 ± 1.8 nmol/mg protein) BSO treatment alone significantly decreased GSH (9.6 ± 2.1 nmol/mg protein; $P < 0.001$) but co-incubation with 15d-PGJ2 for 24hours prevented the

depletion elicited by BSO (21.3 ± 2.7 nmol/mg protein). Compared to untreated cells BSO treatment decreased IL-6 secretion (from 0.94 1.6ng/ml to 0.69 ± 1.3 ng/ml) and total Nrf2 protein levels (by 21%). Co-incubation with 15d-PGJ2 for 24 hours with BSO did not change IL-6 (0.67 ± 1.4 ng/ml) or total Nrf2 level at any time point.

This study suggests that neuronal toxicity resulting from glutathione depletion can be restored by the induction of Nrf2-ARE pathway and the role of the Nrf2 signalling merits further investigation in neurodegenerative diseases.

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PP35

Lipid peroxidation as a consequence of diabetes: effects of different treatment

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The epidemiological studies have shown that type 2 diabetes leads to oxidative stress formation. Therefore the aim of this study has been to estimate the lipid peroxidation in type 2 patients and 50 patients (33 woman and 17 man) that were treated with insulin and antidiabetic drugs and 50 healthy subjects (35 woman and 15 man) were included into the study. Plasma of diabetes patients was characterized by enhanced activity of GSH-Px and Cu,Zn-SOD and lack of changes in GSSG-R in comparison to healthy people with no changes between patients groups. However vitamin A, E, C and reduced glutathione levels were diminished in both groups of patients (treated with insulin and antidiabetic drugs) compared to healthy subjects and the largest decrease was observed after insulin administration. Changes in antioxidant defense led to oxidative stress formation that consequence was enhanced lipid peroxidation. Increased level of 4-HNE and 4-ONE and no changes in the level of 4-HHE and MDA were observed in diabetes in comparison to healthy subjects. Significantly higher level of MDA was observed in plasma of patient after treatment with antidiabetic drugs than insulin treatment. However in plasma of diabetic patients treated with insulin and antidiabetic drugs two fold increase in the level of isoprostanes (free and total isoprostanes) and neuroprostanes in comparison to control group were also observed. Above changes were accompanied by significant increase in the activity of PAF-AH and PLA_2 in the plasma of 2 type diabetes patients. Moreover diabetes leads to decrease in the free LA level as well as phospholipid AA and DHA levels and the lowest decrease in LA were observed after treatment with insulin. In conclusion, diabetes is characterized by oxidative stress and estimation of lipid peroxidation may be helpful of pharmacotherapy.

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PP36

Investigating the ability of antibodies to recognize specific oxidized protein epitopes.

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There is a growing awareness that inflammatory diseases have an oxidative pathology, which can result in specific oxidation of amino acids within proteins. Antibody-based techniques for detecting oxidative post-translational modifications (oxPTMs) are often used to identify the level of protein oxidation. There are many commercially available antibodies but some uncertainty to the potential level of cross reactivity they exhibit; moreover little information regarding the specific target epitopes is available. The aim of this work was to investigate the potential of antibodies to distinguish between select peptides with and without oxPTMs. Two peptides, one containing chlorotyrosine (DY-Cl-EDQQKQLC) and the other an unmodified tyrosine (DYEDQQKQLC) were synthesized and complementary anti-sera were produced in sheep using standard procedures. The anti-sera were tested using a half-sandwich ELISA and the anti-serum raised against the chloro-tyrosine containing peptide showed increased binding to the chlorinated peptide, whereas the control anti-serum bound similarly to both peptides. This suggested that antibodies can discriminate between similar peptide sequences with and without an oxidative modification. A peptide (STSYGTGC) and its variants with chlorotyrosine or nitrotyrosine were produced. The anti-sera showed substantially less binding to these alternative peptides than to the original peptides the anti-sera were produced against. Work is ongoing to test commercially available antibodies against the synthetic peptides as a comparison to the anti-sera produced in sheep. In conclusion, the anti-sera were able to distinguish between oxidatively modified and unmodified peptides, and two different sequences around the modification site

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PP37

Discovery of novel photorespiratory players by forward chemical and genetic approaches

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The high metabolic flux through photorespiration constitutes a significant part of the carbon cycle. Although the major enzymatic steps of the photorespiratory pathway are well characterized, little information is available on the functional significance of photorespiration beyond carbon recycling. Particularly important in this respect is the peroxisomal catalase activity which removes photorespiratory H_2O_2 generated during the oxidation of glycolate to glyoxylate, thus maintaining the cellular redox homeostasis governing the perception, integration and execution of stress responses. We have previously performed a chemical screen that led to the identification of 34 small molecules that alleviate the negative effects of photorespiration in Arabidopsis thaliana mutants lacking peroxisomal catalase (*cat2*). In parallel, we screened a mutagenized *cat2* population and looked for second-site mutations that similarly attenuate the effects of photorespiratory conditions in *cat2* plants. Currently, we characterize putative protein targets of some of the chemical hits and investigate the fine-tuning roles of the confirmed mutations on the photorespiratory phenotype of *cat2*.

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PP38

PDIA1 overexpression activates acutely Nox1 NADPH oxidase in VSMC

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Mechanisms regulating NADPH oxidase remain open and include the redox chaperone protein disulfide isomerase (PDI). We described previously that PDI transient overexpression in VSMC enhances basal Nox1 mRNA expression and activity, without any further response for AngII stimulus in ROS production. Here we investigated whether Nox1 activation mediated by PDI overexpression may involve AngII receptor (AT1R).We developed rabbit aortic VSMC with doxicycline inducible system for rat PDI (VSMC-PDI), which showed 3,5 fold increased total PDI expression after 48h of doxicycline addition. VSMC-PDI showed increased superoxide production by NADPH-triggered enriched membrane fraction (1 vs. 2,5 $\pm$ 0,03 RLU/mg protein, lucigenin reduction), which was totally inhibited by Nox1/4 inhibitor (GKT136901, 100uM). VSMC-PDI showed higher migration trajectories vs. VSMC with no stimulus (1 vs. 1,75 $\pm$ 0,04 pixels, end distance from origin by single cell migration assay for 16h). Although inhibitors of AT1R (losartan and candesartan, 10-100nM for 16h) decreased intracellular superoxide (2-hydroxyethidium quantification by HPLC) or extracellular H_2O_2 (AmplexRed assay) productions mediated by PDI transient overexpression, there is no direct PDI interaction with AT1R, based on several confocal microscopy analyzes in both VSMC and HEK293 cells transfected with AT1R-GFP. Since AT1R activates β-arrestin1/2 signaling, we silenced β-arrestin1/2 with further PDI induction in VSMC-PDI. Preliminary results suggest no changes in Nox1 NADPH activity. Finally, chronic VSMC-PDI (obtained after antibiotic selection of VSMC transiently transfected, 4 fold increased PDI expression) showed no more increased intracellular superoxide or Nox1 NADPH oxidase activation compared to basal VSMC. The mechanisms underlying Nox1 acute activation by PDI overexpression in VSMC does not involve direct interaction with AT1R, and might not involve β-arrestin1/2. Chronic PDI overexpression may induce VSMC redox adaptations, since Nox1 NADPH oxidase activity is decayed to basal levels.

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PP39

Indole- and Chromane-type heterocycles: Playing with molecules to defeat ageing

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Heterocyclic compounds with chromane- and indole-type skeleton represent two fairly large groups of substances with proved beneficial antioxidant effects even beyond the nutritional base. Although these compounds have usual origin in the nature, a rational chemical synthesis might offer an efficient tool for "playing" with their physico-chemical properties so as to optimize their biological effects. In the present study, on using the cellular models, we tried to evaluate the potential of chemically modified flavonoids and synthetic hexahydropyridoindoles to ameliorate ageing-related degenerative processes. Introduction of electron-accepting quinonoid substituent into quercetin increased its prooxidant capacity and toxicity, however, at lower concentration, the effects encompassing hormesis were observed. These properties involved enhanced protection of human fibroblast-like cells from oxidative insult as well as anti-inflammatory effects. 3′-O-Acylation of quercetin (ensuring its higher lipophilicity) yielded in enhanced protection of adult rat microglial cells based on suppression of their protein oxidation, downregulation of the cellular senescence markers and induction of a resting, ramified morphology. On the other hand, weakly basic piperidine moiety in pyridoindoles appeared to steer these substances into lysosomal compartments via a proton-trap mechanism, this being followed by their enhanced accumulation. This mechanism yielded in increased staining of the cells, induction of osmotic processes and enhanced protection of the macrophageous cells from oxidative damage. Thus, rational synthetic variations of indole- and chromane-type compounds can facilitate the search for the substances showing enhanced interference in ageing-related pathologies. This is to say, there are numerous modifications susceptible to optimize either specific targeting, bioavailability or electron properties of these hopeful biological molecules [VEGA2/0031/12].

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PP40

Total Antioxidant Capacity (TEAC) is decreased despite high levels of uric acid in patient with atherosclerosis

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Introduction: The atherosclerosis development and plasma antioxidant protection are strongly linked. The intima damage is reflected through the difference between the antioxidants and oxidants in patients with atheromatous plaques. A high level of oxidative stress in atherosclerotic subjects lead to an imbalance in the serum antioxidant capacity.

The purpose of the study was to evaluated the plasma Trolox Antioxidant Capacity (TEAC) and residual antioxidant activity (GAP) in the atherosclerotic patients compared with healthy humans.

Materials and Methods: The study included 33 subjects: 19 patients newly diagnosed with monovascular disease and 14 subjects apparently healthy. The atherosclerotic patients were selected in The Cardiology Department of University and Emergency Hospital, Bucharest. Inclusion criteria for atherosclerotic patients were: recently diagnosed monovascular disease by coronary angiography or high intima media thickness in patients with cardiovascular risk factors. None of the patients were taken statins for more than one week.

The lipid profile: total cholesterol (TC), triglycerides (TG), LDLc, HDLc was determined in EDTA plasma blood after an overnight fast. Uric acid, albumin, TEAC and GAP were also evaluated.

Results: The LDLc, TC, HDLc and TG plasma levels were not statistically significant different between the both groups of study. The uric acid increase was statistically significant in the atherosclerosis group ($p < 0.05$) compared to the control group ($p < 0.05$). There was no significant difference for albumin concentration between groups. TEAC and GAP were statistically significant reduced in atherosclerosis group ($p < 0.05$).

Conclusion: A statistically significant difference was found for TEAC, GAP and uric acid in atherosclerotic patients compared to the controls. The decreased antioxidant capacity may signify a reduced antioxidant protection for this type of patients.

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PP41

Oxygen concentration in oxidative stress and replicative senescence in dental pulp stem cells

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The *in vitro* culture is routinely carried out under ambient oxygen tension. However, *in vivo* human Dental Pulp Stem Cells (hDPSC) are not exposed to such hyperoxic conditions. Dental pulp tissue physiological oxygen tension ranges between values of 3% to 6% O_2. *In vitro* cellular senescence refers to both replicative and premature senescence. Replicative senescence is associated with a progressive increase in the expression of $p16^{INK4a}$. Bmi-1 is a supresor of $p14^{ARF}$ and $p16^{INK4a}$. Premature or accelerated senescence can be induced by ROS accumulation. It has been demonstrated that pluripotency can be induced by introducing 2 factors: Sox2 and Oct4.

The aim of this study was to investigate the involvement of oxidative stress induced by culture under 21% oxygen, in the maintenance of pluripotency and in the mechanisms of chronological and premature aging of hDPSC.

hDPSC cultured under 21% O_2 showed dihydrorhodamine levels significantly higher, and their mitocondrial membrane potential was lower in comparison to hDPSC cultured under 21% O_2. Bmi-1 expression increases with passages, but this expression is higher in hDPSC cultured under 21% O_2 in comparison to 3% O_2 since the beginning. At early passages, Sox2 and Oct4 are overexpressed when culture under 3% O_2 compared to 21% O_2, and its expression decreases with passages. $p14^{ARF}$, $p16^{INK4a}$ expression and beta-Galactosidase activity remain very low in hDPSC cultured under 3% O_2, whereas under 21% O_2 they are significatively augmented. p21 expression increases with passages under both 3% or 21% O_2 culture conditions.

Because hDPSC expressed $p14^{ARF}$ and $p16^{INK4a}$ but not Bmi-1 or p21, we conclude that *in vitro* premature senescence under 21% O_2 may be associated with oxidative stress and not with Bmi-1 overexpression. hDPSC culture under 3% O_2 mantains its stemness and reduces premature senescence.

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PP42

Fisetin regulates TPA-induced breast cancer cell invasion by suppressing matrix metalloproteinase-9 activation via the PKC/ROS/MAPK pathways

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Invasion and metastasis are among the main causes of death in patients with malignant tumors. Fisetin (3,3′,4′,7-tetrahydroxyflavone), a natural flavonoid found in the smoke tree *(Cotinus coggygria),* is known to have antimetastatic effects on prostate and lung cancers; however, the effect of fisetin on breast cancer metastasis is unknown. The aim of this study was to determine the anti-invasive activity of fisetin in human breast cancer cells. Matrix metalloproteinase (MMP)-9 is a major component facilitating the invasion of many cancer tumor cell types, and thus the inhibitory effect of fisetin on MMP-9 expression in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-stimulated human breast cancer cells was investigated in this study. Fisetin significantly attenuated TPA-induced cell invasion in MCF-7 human breast cancer cells, and was found to inhibit the activation of the PKCa/ROS/ERK1/2 and p38 MAPK signaling pathways. This effect was furthermore associated with reduced NF-κB activation, suggesting that the anti-invasive effect of fisetin on MCF-7 cells may result from inhibited TPA activation of NF-κB and reduced TPA activation of PKCα/ROS/ERK1/2 and p38 MAPK signals, ultimately leading to the downregulation of MMP-9 expression. Our findings indicate the role of fisetin in MCF-7 cell invasion, and clarify the underlying molecular mechanisms of this role, suggesting fisetin as a potential chemopreventive agent for breast cancer metastasis.

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PP43

Resveratrol Inhibits Malign Pleural Mesothelioma Cell Proliferation Through Antioxidant System

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Background: Malignant pleural mesothelioma (MPM) is a highly aggressive tumor with no effective cure. Resveratrol (RSV) is a polyphenol present in the grape. Its anti-tumor effects have been reported previously. We investigated antiproliferative effect of RSV on MPM cells and examined if this effect is through antioxidant system.

Method: Two different human MPM cells, epitheloid (NCI-H2452) and biphasic (MSTO-211H) MPM cells, are treated with RSV at different concentrations (5-250 μM) and different exposure times (24h, 48h, 72h) and then antiproliferative effects were determined by WST-1 assay. To figure out if this effect is through ROS production, intracellular ROS were measured by flow cytometry. Furthermore the expressions of antioxidant enzymes were evaluated by Western Blot analysis to explain the mechanism of this effect.

Results: RSV reduced the cell proliferation of both cell lines in a concentration dependent and exposure time- dependent manner compared to control. The ROS levels in the cells were elevated with RSV treatment. The expression of SOD2 increased significantly in biphasic MPM cells by RSV. But we could not find significant changes on SOD2 expression of epitheloid MPM cells with RSV treatment.

Conclusion: These results suggest that the treatment of MPM cells with RSV causes significant inhibition of cell proliferation and this effect is associated with increase of ROS production in the cells. The mechanism of this increased production can be explained with increased SOD2 expression in the cells.

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PP44

Modulation of FoxO1a activity through S-glutathionylation?

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Cysteine-S-glutathionylation is a reversible post-translational modification occurring under oxidative stress. Here, we tested how transcription factor FoxO1a activity is affected by the absence of its cysteine residues and whether glutathionylation of FoxO1a might occur. A V5-tagged cysteine-deficient mutant of human FoxO1a (all 7 cysteines were replaced by serines) was generated by site directed mutagenesis, followed by transfection of HepG2 human hepatoma cells or HEK293 human embryonic kidney cells to overexpress either wild type (WT) or Cys-deficient V5-FoxO1a. Neither insulin-induced phosphorylation of FoxO1a nor its nucleocytoplasmic shuttling was attenuated in cysteine-deficient FoxO1a. Moreover, no alteration of basal FoxO1a DNA binding activity (EMSA) was noticed in the Cys-deficient version versus WT under normal culture conditions. However, exposure to diamide, a thiol-oxidizing agent, revealed that, while FoxO1a-DNA interaction was attenuated in the WT form, this oxidant-induced attenuation was less prominent in the cysteine-deficient mutant. Furthermore, transactivation of a FoxO-responsive element-driven reporter gene was less prominent in cells overexpressing Cys-deficient FoxO1a than in those transfected with WT FoxO1a. Immunoprecipitation of glutathionylated proteins in cells exposed to diamide co-precipitated WT-FoxO1a more efficiently than Cys-deficient FoxO1a. Moreover, Western blotting analyses (reducing/non-reducing) of diamide-exposed cells overexpressing WT or mutant FoxO1a suggest that oxidant-induced FoxO1a interaction with cofactors is attenuated in the Cys-deficient mutant. In summary, these data suggest that cysteine residues in FoxO1a, while not affecting insulin-induced phosphorylation and nucleocytoplasmic shuttling, are important mediators of FoxO1a/DNA interaction under conditions of oxidative stress. Moreover, these data point to glutathionylation of FoxO1a and/or yet to be identified cofactors under exposure to oxidants, suggesting a regulation of FoxO activity through S-glutathionylation.

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PP45

Microwave versus electrical oven heating influence on oxidative stress parameters, flavonoids and polyphenols content of five different commercial cooking oils

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Consumers are increasingly concerned about the health aspects of food preparation. There is data to support that foods treated with microwaves (MW) develop harmful substances. Vegetable oils are susceptible to lipid peroxidation due to the chemical structure, but also contain antioxidant compounds. Peroxidation products can have harmful effects for human health. The aim of the study was to evaluate the variation of total antioxidant capacity and antioxidant degradation in relation to lipid peroxides formation during conventional and microwave oils heating.

Sunflower, corn, soybean, palm and a mixed oil (sunflower, grape, flaxseed and rice oil) were purchased from the local market. To simulate home cooking, different exposure times were tested 5, 10 and 15 min. For all samples, and for each exposure time, Trolox Equivalent Antioxidant Capacity (TEAC), lipid peroxides as thiobarbituric reactive substances (TBARS), conjugated dienes, vitamin E as α-tocopherol, flavonoids and total polyphenols were determined.

After 15 minutes heating the best retention for vitamin E was observed for the palm oil (MW 12 mM/l), and corn oil (convection 17 mM/l). The highest total antioxidant capacity after heating belonged to mixed & palm oil (MW 4.6 eq. TROLOX/l) and soybean oil (convection 7.5 mM eq. TROLOX/l). Highest levels of TBARS belonged to the mixed oil (convection 54 uM/l) and soybean oils (MW 83 uM/l). Highest conjugated dienes levels were observed for soybean oil (MW 17 mM/l) and sunflower oil (convection 22 mM/l). The best retention for polyphenols was found in the mixed and sunflower oils (MW and convection 0.80.9 mg/ml. The retention of flavonoid was very small in all oils and the highest amount was found in corn and soybean oil (0.04 mg/ml).

During the heating of edible vegetable oils commonly used, total antioxidant capacity along with the amount of vitamin E, flavonoids and polyphenols decreases, while the amount of lipid peroxidation products increases and these processes are more intense when using microwave heating than when using convection heating.

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PP46

Antiproliferative activity of prenylated flavonoids from hops against versatile human cancer entities

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Flavonoids form an essential group of secondary plant metabolites, which gained increasing attention due to a broad range of promising health effects described *in vitro* and *in vivo*. The medicinal plant *Humulus lupulus* (hop) contains a large amount of flavonoid derivatives, particularly prenylated flavonoids and chalcones. Compared to "classical flavonoids" little is known about the biological activity of prenylated flavonoids, even though it is suggested that prenylation could even increase their biological activity. We found very interesting anticancer effects, notably for 6-prenylnaringenin (6-PN) and 8-prenylnaringenin (8-PN) from hops and especially beer. This antineoplastic activity was found *in vitro* using cancer cells from prostate cancer (PC-3), renal carcinoma (UO.31), and other aggressive tumour entities including primary patient-derived metastatic tumour cells. According to these findings 6-PN, 8-PN, and other hop-derived prenylflavonoids are currently further evaluated for their possible clinical application.

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PP47

Natural Nrf2 activators protect cells from oxidative damage inflicted by angiotensin II or aldosterone

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The blood pressure regulating hormones angiotensin II (AngII) and aldosterone (Ald) cause oxidative stress and oxidative DNA damage in kidney cells in vitro and in vivo. Strategies to prevent oxidative damage with antioxidants were ineffective in humans. Fortifying the intrinsic cellular antioxidative defense is a new approach to protect cells from oxidative attack.

In kidney cells the effect of the three natural Nrf2 activators curcumin, methysticin and sulforaphane (Sulf) on Nrf2 activation and their impact on Ald-induced DNA damage was studied. Sulforaphane was additionally tested for its potential to prevent AngII-induced DNA damage and NF-kB activation.

All Nrf2 activators were able to protect from Ald-induced DNA damage. Sulf and curcumin led to a significant activation of Nrf2, detected as translocation to the nucleus. These two substances were also able to increase the expression of Nrf2 in a positive feedback manner. Sulf also protected from damage inflicted by AngII. Besides increasing the expression of the antioxidative protein HO-1, Sulf also prevented activation of the pro-inflammatory transcription factor NF-kB.

In conclusion, all Nrf2 activators efficiently protected from Ald- and AngII-induced oxidative DNA damage. The role of the anti-inflammatory effect of Sulf in the prevention of genomic damage needs further investigation.

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PP48

Prevention of peroxynitrite-induced nitration and oxidation by nitroxides

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Peroxynitrite, formed in vivo in a reaction between nitric oxide and superoxide radical anion, is a compound of profound pathophysiological importance. The formation of peroxynitrite is intensified in many diseases, including neurodegenerative ones, and its reactions appear to contribute to the development of these diseases. Therefore, prevention of reactions of peroxynitrite may be useful in disease prevention and treatment. The aim of this study was to study the ability of nitroxides of various structures to prevent reactions of nitration and oxidation of model compounds and bovine serum albumin. Eleven nitroxides were used: 2,2,6,6-tetramethylpiperidine (TEMPO), 4-hydroxy-TEMPO, 4-amino-TEMPO, 4-oxo-TEMPO, 4-carboxy-TEMPO, 4-cyano-TEMPO, 4-methoxy-TEMPO, 4-acetamido-TEMPO, 4-nonylamido-TEMPO, 3-carbamoyl-PROXYL and 3-carbamoyl-dehydroPROXYL. Nitroxides were most effective in preventing peroxynitrite-induced decay of fluorescein (2 μM), conditioned by fluorescein nitration (IC_{50} of 40.0 ± 0.4 nM, 59.8 ± 0.6 nM and 64.4 ± 1,2 nM for the most effective nitroxides, viz. 4-hydroxy-TEMPO, 4-acetamido-TEMPO and 4-amino-TEMPO, respectively, and 3.72 ± 0.05 μM for the least effective 4-nonylamido-TEMPO). Nitroxides had a biphasic effect on the oxidation of dihydrorhodamine 123 (1 μM) by peroxynitrite, both low and high concentrations having a prooxidant effect. A protection window was observed for micromolar concentrations of nitroxides (maximal protective effect at 17.3 ± 2.3, 40 and 50.3 ± 20.7 μM for TEMPO, 4-hydroxy-TEMPO and 4-amino-TEMPO, respectively and > 400 for Pirolin and 4-nonylamido-TEMPO, the extent of maximal protection being 38-90%. Nitroxides (5 nM – 500 μM) were totally ineffective in preventing peroxynitrite-induced thiol oxidation of bovine serum albumin. These results demonstrate that nitroxides are more effective in preventing nitration than oxidation reactions.

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PP49

Mitochondrial involvement in the formation of lipofuscin

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Cellular aging is accompanied by the intracellular accumulation of oxidized cross-linked protein aggregates. Especially aged postmitotic cells show an accumulation of the protein aggregate lipofuscin. It is known that this protein aggregate is indigestible and has detrimental consequences on cellular function, leading to a decreased proteasomal as well as lysosomal activity and eventually causes cell death. Whereas most consequences of lipofuscin on cellular function are well studied it is still unknown which proteins are involved in its formation. However, one theory explaining the process of lipofuscinogenesis is the "mitochondrial-lysosomal axis theory of aging". According to this theory, mitochondria play a crucial role in the formation of lipofuscin and aging in general. Particularly the incomplete degradation of mitochondria via autophagy (mitophagy) seems to be responsible for increased oxidative stress and lipofuscin formation during aging. We examined the influence of mitophagy inhibition, downregulation of mitochondrial lon protease and the impact of mitochondrial targeted antioxidants on lipofuscin formation.

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PP50

Polyphenols protect against SIN-1 induced nitration of cellular proteins

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Peroxynitrite anion ($ONOO^-$) is a potent oxidizing and nitrating agent. A primary effect of $ONOO^-$ on proteins is nitration of tyrosine residues. Nitration of tyrosine residues has been implicated in the pathophysiology of various, particularly neurodegenerative, disorders and aging, therefore search for agents preventing or limiting protein modifications by peroxynitrite is of interest. Our previous study has identified compounds, mostly polyphenols, the most reactive with peroxynitrite. However, protective effects in cell-free systems may be not relevant for the cellular effects. The aim of this study was to study the protective effects of chosen compounds, mainly polyphenols, on the nitration of intracellular proteins. MCF-7 cells were preincubated with chosen antioxidants for 1-5 h. Excess of antioxidants was washed off and the cells were exposed to 3-morpholinosydnonimine N-ethylcarbamide (SIN-1) as a source of peroxynitrite at 37°C for 1 h. The concentration of SIN-1 causing a loss of cell viability (estimated with MTT) to 60% in the absence of any antioxidant was chosen (100 μM). From 25 compounds studied, 9 providing the best protection of SIN-1 exposed cells were chosen for protection against nitration of intracellular proteins. The cells were preincubated with the compounds studied at non-cytotoxic concentration of 1 μM for 5 h and subjected to treatment with SIN-1 for 1 h. The level of 3-nitrotyrosine in cellular proteins was estimated by ELISA. The sequence of effectivity in protection against nitration of intracellular proteins was as follows: ferulic acid > uric acid > desferrioxamine > naringin hydrate > luteolin > genistein > quercetin. This sequence is different from that found in a cell-free system of tyrosine nitration, confirming that other factors, apart from reactivity with peroxynitrite alone, related to the cellular behavior of antioxidants, are important for their protective efficiency against nitration *in situ*.

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PP51

Haemodialysis treatment influence on carbonyl stress parameters

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Chronic renal failure is associated with increased oxidative and carbonyl stress. Even if haemodialysis eliminates toxic metabolic products, the procedure per se is associated with further increased oxidative and carbonyl stress in patients with renal disease. The aim of our work was to evaluate the influence of long term haemodialysis treatment on oxidative and carbonyl stress parameters in patients with chronic renal failure.

Methods: A total of 58 subjects participated in this study: 16 patients with chronic renal insufficiency (CRF) randomly selected from Carol Davila Hospital patients, 20 patients with end stage renal disease undergoing haemodialysis treatment 3 times weekly (HD) and 22 subjects apparently healthy (C) as controls. On blood samples collected after overnight fasting we have determined the concentrations of Amadori Products (AP), total dicarbonilic compounds (DC), malondialdehyde (MDA) and total thiols (SH).

Results: AP, MDA and DC were significantly increased in patients with chronic renal failure (CRF) while total thiols levels were significantly decreased compared with the controls. Interestingly, for patients undertaking haemodialysis treatment (HD) the AP levels were not significantly increased compared with controls while SH levels were increased. MDA and DC levels were also significantly increased in HD patients compared with the controls. We have not found any significant correlation between AP levels and DC levels in our subjects.

Conclusion: Haemodialysis treatment is lowering Amadori Products levels in end stage renal disease patients while having no beneficial effect on total dicarbonilic compounds levels. Increased total thiols levels found in HD patients may constitute an activated antioxidant answer to increased oxidative stress

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PP52

A New Protocol for the Evaluation of the Antioxidant Activity of Plant Components

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A new approach is proposed to evaluate antioxidant activity. It is based on simultaneous measurement of cellular turbidity and hemoglobin. Human erythrocytes were pretreated separately with ginger oleoresin, ginger essential oil, and ascorbic acid. Untreated cells served as control. Oxidative stress was induced by H_2O_2. Samples were then evaluated by simultaneous measurement of cellular turbidity and the released hemoglobin. Additionally, morphological changes of erythrocytes, catalase activity, and lipid peroxydation were investigated. The results showed that the hemoglobin concentrations were significantly higher in samples treated with ginger extracts compared to the control. Surprisingly, cell concentrations were also higher in these same samples. This indicates that in the presence of antioxidants the measurement of hemoglobin levels alone is not a sufficient indicator of hemolysis. These findings were supported by the measurement of catalase activity and lipid peroxidation. We show that a concurrent measurement of erythrocyte concentration and hemoglobin levels is essential in such assays and provide a new protocol that is based on simultaneous measurement of cellular turbidity and hemoglobin levels.

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PP53

Macroautophagy overexpression leads to oxidized proteins accumulation after a short time of oxidative stress

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Oxidative stress has been implicated in several age related diseases and even ageing itself. OS leads to protein carbonylation, a non-enzymatic irreversible event and common feature of aged cells. Carbonylated proteins are dysfunctional and can accumulate in the form of insoluble protein aggregates that alter cellular functionality To cope with carbonylated proteins, cells employ the proteasome, the main non-lysosomal structure for carbonylated proteins turnover. However, if the degrading rate is inferior to carbonylated proteins formation rate, protein aggregates form.

Recently, we found macroautophagy to be upregulated upon protein aggregates formation and proteasome inhibition, possibly as a compensatory mechanism.

In fact, some studies have shown a crosstalk between the proteasome and the lysosomal macroautophagy, however, how this process is actually regulated remains elusive. Being the two main degradative systems in cells, makes it relevant to understand how they cooperate upon oxidized proteins formation.

Taking advantage of our pre-established stress model, we decided to verify if macroautophagy overexpression would compensate proteasome inhibition, and contribute to oxidized proteins clearance.

Preliminary results showed successfully autophagy overexpressing cells using a plasmid for LC3B, an important protein for the process. Confirmation was achieved by Western Blot and immunocytochemistry using also other autophagy markers (such as p62 and atg5) besides LC3. Interestingly, preliminary results also showed that after 3h of oxidative stress, autophagy overexpressing cells exhibited almost the double in carbonylated protein content, comparing to non-transfected cells. This may reflect the proteasome autophagy axis hypothesis. However, further studies should follow to confirm the crosstalk between the two major systems for carbonylated proteins degradation and to understand how they are regulated upon their formation.

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PP54

Oxidative stress in blood plasma of patients with community-acquired pneumonia

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Aim: The main purpose of our investigation was to study the concentration of oxidized proteins and malondialdehyde (MDA) in blood plasma from patients with community-acquired pneumonia (CAP).

Methods: Patients were divided into 2 groups. 29 patients with community-acquired pneumonia moderate severity and respiratory insufficiency of grade 2 were included in the 1-st group. The control group consisted of 32 healthy persons. All patients and healthy subjects had received the full information on probable inconveniences at the blood sampling before giving their written informed consent.

The protein reactive carbonyl derivatives, advanced oxidative proteins products (AOPP), and malondialdehyde (MDA) were detected in blood plasma. Comparisons of the results obtained between patients and healthy persons were performed using non-parametric Mann-Whitney U-test (for independent variables).

Results: Our results showed the significant increase in AOPP (by 2 times, $p < 0.01$), and MDA (by 3.7 times, $p < 0.01$) in blood plasma from CAP patients in comparison with healthy persons. The insignificant alteration of protein reactive carbonyl derivatives in blood plasma from CAP patients was observed.

Conclusion: The data showed synchronous increase of AOPP and MDA in blood plasma from CAP patients. AOPP is considered to be formed from oxidized albumin, fibrinogen. The probability of reaction of AOPP with MDA and cross-linking increases. It promotes oxidative stress over time and contributes to hypoxia progression in CAP patients.

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PP55

Oxidized proteins in neutrophils from patients with community-acquired pneumonia

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Aim: Oxidized proteins are considered as oxidative stress markers. The role of different oxidaized proteins in the progression of community-acquired pneumonia (CAP) might be of great importance. The aim of the present study was to evaluate advanced oxidation proteins products (AOPPs) and protein reactive carbonyl derivatives concentrations in neutrophils from CAP patients.

Methods: 29 patients with community-acquired pneumonia moderate severity and respiratory insufficiency of grade 2 were included in the 1-st group. Patients were observed in a hospital during our research. The control group consisted of 32 healthy persons. All patients and healthy subjects had received the full information on probable inconveniences and complications at the blood sampling before giving their consent to participate. The protein reactive carbonyl derivatives were detected following the protocol of Levine R.L. et al. The AOPPs was measured in neutrophils following the protocol of Witko-Sarsat et al.

Results: There was significant difference between the AOPPs concentrations in neutrophils from CAP patients and the control group. AOPPs concentration in plasma of patients was higher compared with healthy ones (by 2.3 times, $p < 0.001$). We also observed the increasing of protein reactive carbonyl derivatives in neutrophils from CAP patients.

Conclusion: Our results showed significant accumulation of AOPPs in neutrophils from CAP patients. We supposed that protein reactive carbonyl derivatives accumulation in neutrophils might be associated with further progression of pneumonia. In any case, accumulation of oxidized proteins might be connected with decrease of the proteolytic degradation of damaged proteins. In neutrophils excess accumulation of oxidized proteins could lead to misfolding and functional disorders of intracellular proteins, participating in cytoskeletal architecture, transport, redox homeostasis. It might be new insight systemic mechanism of CAP progression.

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PP56

Six-week supplementation with highly bioavailable curcuminoids is safe but does not alter blood lipids and inflammation markers in humans

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Cardiovascular diseases (CVD) are one of the leading risk factors for morbidity and mortality in the Western world. Due to the fact that conventional medications possess side effects, there is large interest to find alternatives.

Curcuminoids are lipophilic phytochemicals from the plant *curcuma longa*. Besides their reported antioxidant, anticancer and anti-inflammatory activities, they may also be able to decrease blood cholesterol when regularly consumed.

Therefore, we investigated, in a randomized, double-blind, crossover study, the effects of curcuminoids, in form of a bioavailability-improved formulation (micelle), on lipid and inflammation markers. Subjects (25 women, 17 men) with moderately elevated concentrations of total cholesterol, LDL-cholesterol and C-reactive protein (CRP) consumed 240 mg curcuminoids/d as micelles or placebo for six weeks, respectively, interrupted by a four week washout phase. Blood was collected at the beginning, after three and six weeks of each intervention and concentrations of curcuminoids, triacylglycerols, total, HDL- and LDL-cholesterol, CRP, interleukine-6 and liver and kidney function markers were determined. In spite of detectable plasma curcuminoid concentrations, lipid and inflammation parameters were not altered by curcuminoid supplementation and safety markers remained in the reference ranges. Thus, daily supplementation of otherwise healthy subjects with slightly elevated blood lipids and inflammation markers with highly bioavailable curcuminoids is safe, but does not alter blood lipids and inflammatory status.

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PP57

Examination of the Antioxidant capacity of water extracts of common teas using the Cellular Antioxidant Activity (CAA) assay

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Oxidative stress leads to a high production of free radicals, which are related with more than a hundred of human diseases, including cancer, atherosclerosis and Alzheimer's, and may be important in the process of ageing. Natural products rich in antioxidant compounds can neutralize *in vitro* the potentially harmful free radicals, suggesting that an antioxidants-rich diet might provide health benefits.

Several *in vitro* methods have been developed to test the antioxidant potential of foods. Though, these assays do not evaluate the antioxidants in a physiological environment and do not take consideration of the complexity of a biological system.

The goal of this study was to investigate the antioxidant activity of water extracts of commercial teas within a cellular model. The antioxidant activity was determined using the peroxyl radical scavenging capacity (PSC) and the oxygen radical absorbance capacity (ORAC) methods and compared with the *in vivo* results obtained with the cellular antioxidant activity (CAA) assay.

The results of this study showed that despite some extracts have a relative high ranking of antioxidant potential, this rank is dependent on the chemical method used. Furthermore, the chemical results are weakly correlated with the cell-based method.

Considering the fact that antioxidants are effective in preventing various chronic diseases, the potential antioxidant role of dietary antioxidants within a cellular model certainly merits further attention. The CAA assay takes into account some key biological parameters that have to be considered to estimate the *in vivo* potential result.

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PP58

Oxidative Stress and Ubiquitin Ligases: their involvement in skeletal muscle atrophy

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Introduction: Muscle atrophy plays a relevant role in the many very prevalent diseases. Generation of reactive oxygen species (mainly by the xanthine oxidase) and inflammation are two of the main triggers of muscle atrophy.

Aim: The major aim of our study was to determine the mechanism by which reactive oxygen species activate E3 ubiquitin ligases (MuRF-1 and MAFbx) cause muscle atrophy. Possible prevention by allopurinol, a well-known xanthine oxidase inhibitor widely used in clinical practice; and by indomethacin, a non-steroidal antiinflamatory drug was also studied.

Materials and methods: Male C57BL/6J mice (3 months old) conditioned by 14 days of hindlimb unloading with or without each treatment or the combination of both of them (n=48) were compared with freely ambulating controls (n=48).

Results: After the experimental intervention, we found that hindlimb unloading induced a significant increase in xanthine oxidase activity and prostaglandins in plasma (209%, $p < 0.001$ and 114%, $p < 0.05$; respectively). The most relevant new fact reported is that the combination of allopurinol and indomethacin prevents soleus muscle atrophy (from -41% to -16%, $p < 0.001$). This is mediated by the inhibition of the E3 ubiquitin ligases MAFbx, MuRF-1 and Cbl-b, related to the inhibition of p38 MAPK and NF-κB and the stimulation of Akt pathways respectively.

Conclusions: Our data point out the potential benefit of allopurinol and indomethacin administration for bedridden, astronauts or muscle disuse; as well as a potential benefit in other atrophy models such as pathology-related cachexia or sarcopenia.

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PP59

Oxidative Stress And Ubiquitin Ligases: Their Involvement In Alzheimer's Disease Pathophysiology

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Oxidative stress is a major hallmark in Alzheimer's Disease. We showed that amyloid beta ($A\beta_{1-42}$), induces mitochondrial oxidative stress. We focused on dysregulations of ubiquitin ligases in Alzheimer's and their relation to oxidative stress. The anaphase-promoting complex/cyclosome (APC/C)-Cdh1 ubiquitin ligase has a role as cell cycle regulator in proliferating cells and, recently another role in the regulation the degradation of key glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase-3 has been found (Almeida et al., 2012).

Herrero-Mendez et al. observed in 2009 that inhibition of Cdh1 leads to an upregulation of Pfkfb3 in neurons and that this results in the activation of glycolysis and a lowering of the pentose phosphate pathway. Normally, APC/C-Cdh1is involved in the down-regulation of glycolysis in neurons. They use glucose to maintain their antioxidant status (through utilization of glucose in the pentose phosphate pathway, a metabolic route involved in the regeneration of reduced glutathione) at the expense of its utilization for bioenergetic purposes.

We show that Aβ treatment decreases the protein level of cdh1 in primary neurons in culture. Aβ treatment, cdh1 silencing using siRNA or direct APC/C inhibition using proTAME, all lead to accumulation of APC/C-Cdh1 degradation targets, e.g. cyclin B1 or glutaminase. Glutaminase converts glutamine to glutamate, a very important neurotransmitters in the brain. We observed increased levels of the glutamate concentration in the extracellular medium and subsequently to higher intracellular Ca^{2+} levels inside neurons upon Aβ treatment. This activates cdk5, a kinase that phosphorylates cdh1 and that causes further deactivation of APC/C, entering a positive feedback loop of glutamate generation. Excitotoxicity, induced by high levels of glutamate, has been related to oxidative stress in neurons (Chen et al., 2013). Thus this pathway could be an important common link of oxidative stress and ubiquitin ligases in Alzheimer's Disease.

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PP60

Comparative Study of the Antioxidant Properties of *Stevia rebaudiana* using cellular approaches

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In recent years, *Stevia rebaudiana* is gaining popularity in Europe, especially in weight control and among diabetics since it has a high sweetening power without calories. More recently, its antioxidant properties have been studied with different chemical methods; all authors have agreed that stevia has antioxidant activity.

Our study aims to better examine its antioxidant properties using a relevant model to assess the antioxidant capability of stevia extracts within human cells, to determine its efficiency of protection against free radicals under physiological conditions. To this end, stevia leaf and stem samples were subjected to water extraction, chemically characterized and subsequently the antioxidant potentials were determined *in vitro* through the ORAC assay and *in vivo* in a cell-based assay (CAA). The CAA assay measures ROS levels in the presence or absence of stevia extracts using 2′,7′-dichlorofluorescein diacetate dye, a molecule that becomes a highly fluorescent probe in the presence of oxidants. In addition, purified steviol glycosides were analyzed.

Our results showed that stevia extracts decreased the intracellular oxidation in a dose-dependent manner when compared to control cells, indicating its antioxidant action within the cell. Despite, its purified steviolglycosides showed a low ORAC value compared with crude extracts and do not elicit any cellular antioxidant activity. This study provides evidence that stevia is certainly a potential source of antioxidative agents for the food and dietary supplements companies, that apart its natural sweetness has a cellular antioxidant potential. Moreover, the antioxidant capacity both *in vitro* as well as in the cell-based assay is higher for crude leaves extracts than for purified steviol glycosides, thus suggesting that the polyphenols present in the crude extracts are responsible for the antioxidant activity of stevia. Thus, stevia leaves or crude extracts thereof might be considered as a food ingredient.

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PP61

Protective effect of lingonberry and blueberry extracts on rat brain cells exposed to oxidative stress

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Berry fruits are known for their high antioxidant potential pertaining to their high phenolic content. Antioxidant capacities and phenolic content of blueberry and lingonberry fruits and leaves were studied. Concentrations of total flavonoids, tannins, proanthocyanidin as well as reduced and oxidized levels of ascorbate and glutathione were also determined in these extracts. This study also determined the potential neuroprotective effect of extracts from fruits and leaves against glutamate-mediated excitotoxicity, which is believed to contribute to disorders such as stroke and neurodegenerative diseases. Brain-derived cell cultures from rats were prepared and grown for about 2 weeks. Cell cultures were treated with glutamate (0.1 mM) for 24 h, and the effect of extracts was determined on cells subjected to this excitotoxicity. Blueberry fruits and leaves from both the extracts showed a significant neuroprotective effect. In this study we have shown how phenolic compounds as well as reduced and oxidized levels of ascorbate and glutathione correlate with neuroprotective effect.

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PP62

Manipulation of Environmental Oxygen Modifies Reactive Oxygen Species Generation during Myogenesis

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Regulated changes in ROS formation are important in myogenesis. Both excessive formation and reduction in ROS affect muscle differentiation in a negative way. Cultured cells are typically grown in 20% O_2, but this is not physiological for a number of cell types, including skeletal muscle. The aim was to examine the generation of ROS in cultured skeletal muscle cells under more physiological conditions (6% O_2) and determine whether this affects muscle myogenesis. Primary muscle cells were cultured in 35mm gelatin coated tissue culture plates in DMEM containing 20% (v/v) FCS. To induce myotube formation the medium was replaced with DMEM containing 2% horse serum. Cultures were grown in 20% or 6% O_2 environments throughout myogenesis and ROS were monitored at different stages of myogenesis using dihydroethidium (DHE) and other fluorescent probes. Data demonstrate that proliferation of satellite cells was increased when cells were grown in 6% compared with 20% O_2. Myoblasts grown in 20% O_2 showed an increase in DHE oxidation compared with myoblasts grown in 6% O_2 (1764.1 ± 141.9 vs 1003.7 ± 124.6 relative fluorescence units [RFU]). Myotubes differentiated in 20% O_2 also showed an increase in DHE oxidation compared with myotubes grown in 6% O_2 (2337.6 ± 155.5 vs 1712.9 ± 110.0 RFU). These data indicate that the oxidation of DHE (reflecting superoxide activity) in resting skeletal muscle myoblasts and myotubes is influenced by changes in environmental oxygen concentration and associated with inhibited myogenesis.

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PP63

Xanthine oxidase regulates pesticides-induced oxidative stress in rat polymorphonuclear leukocytes via NOS-independent pathway

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Oxidative stress is reported to be a key malefactor in pesticides-induced toxicity. Xanthine oxidase (XO) is implicated in pesticides-induced oxidative stress and nitric oxide (NO) plays a decisive role in XO-mediated toxicity. The study aimed to investigate the role of XO in pesticides-induced (combined maneb/MB and paraquat/PQ-induced) oxidative stress in polymorphonuclear leucocytes (PMNs) and its subsequent relationship with inducible NO synthase (iNOS)-mediated nitrosative stress. Male Wistar rats were administered with MB and PQ, twice a week, for 2 weeks along with vehicles. In a few sets, rats were also treated with aminoguanidine/AG (an iNOS inhibitor), allopurinol/AP (a XO inhibitor), pyrrolidine dithiocarbamates/PDTC (a NF-κB inhibitor), pentoxyphylline/PTX (a TNF-α inhibitor) and dexamethasone (an anti-inflammatory drug) along with respective controls. While pesticides augmented reactive oxygen species (ROS), lipid peroxidation (LPO) and nitrite production, iNOS expression and superoxide dismutase (SOD) and XO activity, catalase activity was attenuated. AP ameliorated pesticides-induced changes in ROS, LPO, SOD, catalase and XO however nitrite content and iNOS remained unaltered. AG, on the other hand, alleviated pesticides-induced alterations in LPO, nitrite, catalase and iNOS in the PMNs but ROS, SOD and XO were found to be unchanged. Furthermore, PDTC, PTX and DEX also attenuated pesticides-induced increase in XO expression in the PMNs. The results demonstrate that XO mediates pesticides-induced oxidative stress in the PMNs through iNOS-independent mechanism, which might be regulated by inflammatory cytokines.

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PP64

Nrf2-mediated protection by the copper-bis(thiosemicarbazone) complex $Cu^{(II)}$ATSM in human vascular smooth muscle cells

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Copper(II)-diacetyl-bis(N4-methylthiosemi-carbazone) [$Cu^{(II)}$ATSM] was developed as a selective positron emission tomography agent for imaging hypoxia, however the mechanism of its intracellular retention remains to be elucidated. Recent studies have highlighted the neuroprotective effects of $Cu^{(II)}$ATSM against nitrosative damage in a mouse model of amyotrophic lateral sclerosis. As $Cu^{(II)}$ATSM has also been shown to reduce peroxynitrite levels and oxidative stress *in vitro*, we sought to assess the effects of $Cu^{(II)}$ATSM on activation of the Nrf2 defence pathway and protection against angiotensin II (Ang II) mediated apoptosis in human coronary artery smooth muscle cells (HCASMC). Treatment of HCASMC with Ang II (200nM, 12h) significantly increased apoptosis detected by annexin V fluorescence ($P < 0.001$, $n = 5$). Pre-treatment of cells with $Cu^{(II)}$ATSM (1mM, 12h), prior to Ang II (200nM, 12h) significantly attenuated Ang II elicited apoptosis. To assess the effect of $Cu^{(II)}$ATSM on antioxidant protein expression, cells were treated with either vehicle (0.01% DMSO) or $Cu^{(II)}$ATSM (12h, 100 nM, 500nM, and 1μM) and cell lysates analysed by immunoblotting for heme oxygenase-1 (HO-1) and peroxiredoxin 1 (prx1). A significant increase in HO-1 (3-fold), and Prx1 (2-fold) expression was observed following treatment with 1μM $Cu^{(II)}$ATSM compared to vehicle treated cells ($P < 0.05$, $n = 4$). Silencing of Nrf2 in HCASMC attenuated $Cu^{(II)}$ATSM mediated increases in HO-1 and Prx-1 protein expression. Furthermore, treatment of HCASMC with $Cu^{(II)}$ATSM (30 min, 1μM) also significantly increased phosphorylation of Nrf2 at serine 40 (2.4-fold, $P < 0.01$, $n = 4$). Immunofluorescence studies revealed enhanced nuclear levels of Nrf2 following treatment with $Cu^{(II)}$ATSM (4h, 1μM). Thus, we demonstrate that $Cu^{(II)}$ATSM mediates protection of HCASMC via Nrf2 activation and therefore suggest that $Cu^{(II)}$ATSM may provide a potential therapeutic strategy against redox stress in cardiovascular diseases.

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PP65

Regulation of cell death receptor S-nitrosylation and apoptotic signaling by Sorafenib in hepatoblastoma cells

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Nitric oxide (NO) plays a relevant role during cell death regulation that limits the survival of tumor cells. We have recently shown that the overexpression of nitric oxide synthase type III (NOS-3) induces oxidative and nitrosative stress, p53 and cell death receptor expression and apoptosis in hepatoma cells. Sorafenib is the unique recommended molecular-targeted drug for the treatment of patients with advanced hepatocellular carcinoma. The present study was addressed to elucidate the potential role of NO during Sorafenib-induced cell death in hepatoblastoma cells. We determined the intra- and extracellular NO concentration, cell death receptor expression and their S-nitrosylation modifications, and apoptotic markers in Sorafenib-treated HepG2 cells. The effect of NO donors on above parameters has also been determined. Sorafenib induced cell death in HepG2 cells. However, low concentration of the drug (10 nM) increased the expression of cell death receptors and extrinsic apoptotic pathway (caspase-8) that both diminished at higher concentrations of the drug (10 μM). High doses of Sorafenib correlated to a rise of caspase-9 and caspase-3 activities, as well as DNA fragmentation in HepG2 cells. The shift of cell death signaling pathway was associated with a reduction of S-nitrosylation of cell death receptors in cells treated with Sorafenib. The administration of NO donors increased S-nitrosylation of cell death receptors and overall induction of cell death markers in control and Sorafenib-treated cells. In conclusion, Sorafenib induced alteration of cell death receptor S-nitrosylation status which may have a relevant repercussion on cell death shift from the extrinsic to intrinsic apoptotic signaling in hepatoblastoma cells.

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PP66

7-keto-cholesterol: a free radical-derived oxysterol of human adipose tissue with anti-adipogenic activity

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Aims: Oxidative stress of adipose tissue (AT) emerges as an instigator of metabolic dysfunctions in obese insulin-resistant patients. Here we test the hypothesis whether free radical-derived cholesterol oxidation products (i.e., oxysterols) are: 1) generated in the dysfunctional "diabetic" AT, 2) secreted in AT interstitial fluid, 3) and active as modulators of AT stem cell (ASC) adipogenic differentiation.

7-ketocholesterol (7k-C) and 7β-hydroxycholesterol (7β OH-C) were assessed in AT microdialysis samples of healthy subjects as well as in subcutaneous abdominal AT of type 2 diabetic obese patients. Adipogenic differentiation and "canonical" Wnt and MAPK signaling were investigated in primary cultures of human ASCs treated with 7k-C or with the autoxidation-type II oxysterol 5,6-secosterol (5,6-S) for comparison.

Results: 7k-C and 7β OH-C were detected in human AT interstitial fluid (0.27 ± 0.03 and 0.91 ± 0.13 μM, respectively). When compared with non-diabetic individuals, diabetic obese patients showed increased levels of 7k-C and 7β OH-C, as well as of 4-HNE-protein adducts, in subcutaneous AT. Challenging ASCs with 7k-C at levels resembling those found *in vivo* (1 μM), resulted in a lowered adipogenic differentiation by the transient increase of cellular ROS and sequential activation of Wnt/β-catenin, ERK1/2, p38-MAPK and JNK signaling. Signaling data differentiated the anti-adipogenic response of 7k-C and 5,6-S.

Innovation and Conclusion: Free radical-derived oxysterols were identified in the human AT secretome and may act as novel adipokines with regulatory effects on ASC differentiation. Pathogenic effects of these molecules and particularly of 7k-C in the impaired *de novo* adipogenesis of insulin-resistant patients is worth of further investigation.

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PP67

Serum α-Tocopherol Has a Nonlinear Inverse Association with Periodontitis among US Adults

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Previous experimental models suggest that vitamin E may ameliorate periodontitis. However, epidemiologic studies show inconsistent evidence in supporting this plausible association.

We aimed to investigate the association between serum α-tocopherol (αT) and γ-tocopherol (γT) and periodontitis in a large cross-sectional US population.

This study included 4708 participants in the 1999–2001 NHANES. Serum tocopherols were measured by HPLC and values were adjusted by total cholesterol (TC). Periodontal status was assessed by mean clinical attachment loss (CAL) and probing pocket depth (PPD). Total periodontitis (TPD) was defined as the sum of mild, moderate, and severe periodontitis. All measurements were performed by NHANES.

Means ± SDs of serum αT:TC ratio from low to high quartiles were 4.0 ± 0.4, 4.8 ± 0.2, 5.7 ± 0.4, and 9.1 ± 2.7 μmol/mmol. In multivariate regression models, αT:TC quartiles were inversely associated with mean CAL (P-trend = 0.06), mean PPD (P-trend < 0.001), and TPD (P-trend < 0.001) overall. Adjusted mean differences (95% CIs) between the first and fourth quartile of αT:TC were 0.12 mm (0.03, 0.20; P-difference = 0.005) for mean CAL and 0.12 mm (0.06, 0.17; P < 0.001) for mean PPD, whereas corresponding OR for TPD was 1.65 (95% CI: 1.26, 2.16; P-difference = 0.001). In a dose-response analysis, a clear inverse association between αT:TC and mean CAL, mean PPD, and TPD was observed among participants with relatively low αT:TC. No differences were seen in participants with higher αT:

TC ratios. Participants with γT:TC ratio in the interquartile range showed a significantly lower mean PPD than those in the highest quartile.

A nonlinear inverse association was observed between serum αT and severity of periodontitis, which was restricted to adults with normal but relatively low αT status. These findings warrant further confirmation in longitudinal or intervention settings.

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PP68

Cytochrome P450 metabolism of vitamin E in models of non-alcoholic fatty liver disease and steatohepatitis

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Non-alcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease worldwide in association with oxidative stress and insulin resistance. Steatosis can evolve to steatohepatitis (NASH) by lipotoxicity, inflammation, fibrosis and cell death leading to cirrhosis and hepatocellular carcinoma. No valid therapy has been developed so far to control inflammatory and fibrogenic pathways of NAFLD/NASH.

α-Tocopherol (α-TOH) is an antioxidant that partially reverts the histopatological spectrum of NASH. However, the biological mechanism that may support a preventive or therapeutic role of vitamin E in NASH and chronic liver diseases remain elusive. In this study we explored *in vitro* and *in vivo* the metabolism of vitamin E in liver cells and in the whole liver exposed to some pathogenic stimuli associated with the progression of NAFLD/NASH. Protein levels of CYP4F2, the cytochrome P450 isoenzyme involved in the metabolism of vitamin E increased in HepG2 cells incubated with α-TOH. This effect increased with a synergic action by the treatment with the fatty acids oleic acid or palmitic acid, or with fructose, which are well-known NASH stimuli. In mice fed control or NAFLD/NASH diets, e.g. high-fat (HFD) or HFD plus fructose diet, the abnormal lipid metabolism of liver tissue was associated with a lowered expression of CYP4F2 protein. The same finding was observed in animals treated with CCl4, a free radical-generating hepatotoxic xenobiotic leading to hepatic fibrosis, in the presence of lowered levels of liver α-TOH.

In conclusion, either acute or chronic challenges with NAFLD/NASH stimuli influence the liver expression of CYP4F2. The observed acute transcriptional upregulation and the *in vivo* downregulation of this protein, point to a role for CYP4F2 in the inflammatory and lipotoxicity signaling of NASH. Transcriptional effects of vitamin E on this gene may help to explain earlier and positive clinical findings, which are worth of further investigation.

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PP69

High-glucose-induced impairment of the proteasomal system in pancreatic Min6 cells

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Protein degradation by the 26S proteasome is a fundamental process involved in a broad range of cellular activities, yet how proteasome activity is regulated remains poorly understood. In particular, the mechanisms of redox-dependent regulation of the ubiquitin-proteasomal system (UPS) are not clear. High glucose has been proposed to promote oxidative stress in different cellular models. Min6 b-cells grown under high glucose conditions showed a decreased 20S (chemotrypsin) activity compared to control cells, while 20S core subunits were increased as determined by western blot. Peroxiredoxin 5 levels were increased under these conditions, suggesting a response to chronic oxidative stress conditions. On the other hand, short-term exposure of the cells to high glucose concentrations caused a decrease in 26S activity and an increase in 20S activity. Similar results were observed when cells were incubated with the mitochondrial uncoupler antimycin A or hydrogen peroxide. Glucose-induced increase of 20S activity was time-dependent and this effect was abolished when the 20S activity was measured in the presence of the thiol reducing agent dithiotreitol. Dimerization of mitochondrial and cytosolic peroxiredoxins evidenced an increase in the steady-state concentration of peroxides under these conditions. Moreover, non-denaturing PAGE followed by in-gel proteasome activity evidenced the formation of aggregates and changes in proteasome structure which were partially reversed by thiol reduction. Our data suggest that high glucose promotes oxidative stress in Min6 b-cells with a concomitant UPS impairment. Further studies will focus on the thiol modifications of the UPS induced under this conditions and its reversibility.

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PP70

Assessing inhibition of macrophage migration inhibitory factor by isothiocyanates

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Isothiocyanates are a class of phytochemicals that occur in cruciferous vegetables, and have been shown to exhibit anti-inflammatory and anti-cancer activity in both cell and animal models. Macrophage migration inhibitory factor (MIF) was discovered as a major target of isothiocyanates. MIF is a highly conserved pro-inflammatory cytokine that has been associated with several human diseases including sepsis, cardiovascular disease and cancer. MIF has an unusually reactive N-terminal proline that bestows the protein with tautomerase activity. Isothiocyanates covalently modify this proline and inhibit MIF activity.

In this study we have used a library of novel isothiocyanates to investigate their ability to inhibit the tautomerase activity of MIF, both with recombinant human MIF and in cell culture. IC_{50} values as low as 0.25 μM were observed in cells. In cellular models of liver fibrosis, we have shown that isothiocyanates also inhibit the biological activity of MIF. There was no correlation between MIF inhibition and isothiocyanate cytotoxicity, suggesting independent pathways. Finally, we have shown that the reactive N-terminal proline makes MIF susceptible to other

biological electrophiles, including epicatechins. This provides a novel mechanism to explain the anti-inflammatory activities of these dietary phytochemicals.

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PP71

NOX, NOS and sGC as new therapeutic targets in stroke

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Ischemic stroke is the second leading cause of death worldwide and the leading cause of disability. Despite this high medical need only a single drug is available but due to its limited time window and risk of bleeding 85% of all patients are excluded from treatment. Here we address three different new targets in stroke highly related to oxidative stress. NADPH oxidase is one of the most important sources of reactive oxygen species, which has been recently considered as a promising target in ischemic stroke. Neuronal nitric oxide synthase (NOS1) has been also suggested as a possible target so that its partial inhibition can lead to neuroprotective effects. Similarly, NOX possibly acts by scavenging or toxifying NO to nitrating species and oxidising the NO receptor, soluble guanylate cyclase (sGC). This then diminishes physiological NO-cGMP signalling, a process that could be reversed in mice by so-called sGC activator compounds. Pharmacological targeting of apo-sGC in vitro under oxygen and glucose deprivation conveyed strong neuroprotection via PKG/ERK/CREB signalling pathway. In vivo, post-stroke apo-sGC activation by two distinct members of this compound class augmented cerebral blood-flow whilst leaving systemic blood pressure unaffected, reduced infarct size and increased survival. Thus, both inhibiting NOX/NOS-dependent oxidative stress and augmenting cGMP signalling are neuroprotective in stroke. Current and future experiments are aimed at validating these finding in a second rodent species for further preclinical and clinical development as first-in-class neuroprotective drugs.

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