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"REDOX HOMEOSTASIS: FROM SIGNALING TO DAMAGE"

Abstracts of poster presenters in alphabetical sequence of first author

9. Unravelling the effect of N(ε)-(carboxymethyl)lysine (CML) and N(ε)-(carboxyethyl)lysine (CEL) on the ability of α-Synuclein to reduce the formation of Cu^{2+}-catalyzed reactive oxygen species

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α-Synuclein (αS) is a protein found in the neurons of the substantia nigra, where plays a role in neurotransmission and maintains the redox balance [1]. Moreover, αS is well-known to be involved in the development of Parkinson's disease (PD) since it forms the PD-associated intraneuronal fibrillary deposits, known as Lewy bodies (LB). Aggregation of αS is stimulated by mutations, metal chelation, oxidative damage and post-translational modifications. In fact, most LB isolated from post-mortem brains are modified through the glycation process. Although the effect of the advanced glycation end products (AGEs) on the aggregation of αS has been studied, there are not data reporting how the AGEs formed on αS affect the neuronal redox equilibrium. Consequently, we have studied how N(ε)-(carboxymethyl)lysine (CML) and N(ε)-(carboxyethyl)lysine (CEL) -two AGEs found in the neurons of PD models [2]- affected the capacity of αS to inhibit the formation of reactive oxygen species (ROS) from Cu^{2+}-catalysed ascorbic acid (AA) degradation, and to be oxidized by ROS. The obtained data clarify the effect on glycation on the capacity of αS to protect against oxidative damage.

[1] Béraud D, et al. J. Neuroimmune Pharmacol., 2013, 8, 94-117.
[2] Choi YG, Lim S. Biochimie, 2010, 92, 1379-1386.

10. Functional evaluation of oxidative stress parameters in hypoxic premature born infants

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Objective: was to evaluate the intensity of oxidative stress in hypoxic premature born infants.

Material and method: Study group: 43 premature infants born in 2017, in „Bega" Hospital Timisoara.The inclusion criteria: gestational age under 32 weeks, Apgar score ≤ 6/ 5 min, lab analysis for metabolic acidosis ratio and clinical signs for hypoxia. The oxidative stress and antioxidant capacity evaluation was perfomed by d-ROM and BAP parameters (Panel Carratelli, Diacron Italy). Results: Values above 300 unit Carr for d-ROM are indicative for oxidative stress, 76% of patients had these values (the maximal level was 465 U Carr). The optimum value for BAP is higher than 2200 microM/l. At birth 28% of cases had a border-line condition for BAP. At 72 hours of life, d-ROM increased in 45% of the cases, but in parallel manner with the increase of BAP. In 8% of the patients, d-ROM remains high and BAP- under the normal level, in these patients the death occurred. Conclusion: This study pointed out a strong correlation between high d-ROM and/or low BAP level and the prognostic for the patient's disease.

11. Protective effects of L-carnitine against radiation-induced surface and gland epithelial degeneration in rat endometrium

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Purpose: The aim of the study was to detect the effects of L-carnitine (LC) on radiation-induced uterine injury.
Materials and methods: Thirty Wistar albino rats were divided into five groups. The control group received physiological saline intraperitoneally. Radiation-1 and radiation-2 groups received whole-body X-irradiation of 8.3 Gy as a single dose. These groups were sacrificed at the 6th hour and on the 4th day after irradiation, respectively. The radiation-1+LC and the radiation-2+LC groups received the same dose irradiation plus a daily dose of 200 mg/kg LC.
Results: The levels of serum MCP-1 and IFN-γ were significantly higher in the radiation groups than the control groups. Treatment with LC decreased the serum MCP-1 and IFN-γ levels considerably. Radiation induced flattening of the endometrial surface and glandular epithelium, depletion of deep glands, effects that were partially blocked by LC treatment. The expression of proinflammatory cytokines in uterine tissue were markedly stimulated in irradiated rats. In the radiation groups, PARP-1, IL-1β, IL-6, TNF-α and NFκB expression in the uterine tissue were

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important player in preventing excessive superoxide accumulation in mitochondrial distress and induction of programmed cell death in kidneys during BEN development.

117. Identification and relative quantification of 3-nitro-tyrosine residues in fibrinogen using liquid chromatography coupled with tandem mass spectrometry. Analysis of ischaemic stroke

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Ischemic stroke, an acute vascular event that interferes with blood supply to the brain, is one of the leading causes of death worldwide. During the acute ischemia, nitric oxide is secreted to favor vasodilation and reperfusion. Reperfusion induces a burst of free radicals such as superoxide anion. $^{\bullet}NO$ reacts with $O_2^{\bullet-}$ producing peroxynitrite anion. The formation of 3-nitrotyrosine is an oxidative protein modification left by $ONOO^-$, causing alterations in structure and function. The nitration of fibrinogen by $ONOO^-$ is a potential biomarker. A mass spectrometry approach was developed to evaluate the nitration of fibrinogen and its implication in ischaemic stroke. A mapping of 3-nitrotyrosine and a relative quantification of it were made. Twenty different tyrosine residues were identified as nitration targets, after treatment with $ONOO^-$. The number of 3-nitrotyrosine residues and the abundance of those showed to vary in a $ONOO^-$ concentration-dependent manner. The fibrinogen chains have different susceptibility to nitration. Nine peptides were found to be more frequently modified and their peptide-specific transitions were chosen to perform a targeted analysis in clinical samples. Three of the nine peptides showed a significative difference between stroke and control. The nitration pattern *in-vitro* is not the same as the one *in-vivo*.

118. Catalase inhibition leads to translocation of glutathione peroxidase to the erythrocyte membrane

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We aimed to study the role of glutathione peroxidase (GPx) on cytosol and membrane of red blood cells (RBCs) when catalase (CAT) is impaired. We performed in vitro assays (n=4) by incubating RBCs from healthy volunteers with increasing H2O2 concentrations (0, 15, 30, 60μM) while inhibiting CAT (sodium azide). The cytosol activity of GPx, reduced and oxidized glutathione ratio (GSH/GSSG) and RBC membrane lipid peroxidation (LPO) were evaluated by spectrophotometry; GPx amount in RBC membrane was determined by western-blot.
In the RBC cytosol, GPx activity decreased as H2O2 concentration increased; simultaneously, we found decreasing GSH/GSSG. At the RBC membrane, we detected increasing linkage of GPx with increasing H2O2 concentrations; LPO values increased when H2O2 was added, however for 30 and 60μM of H2O2 no differences were found.
Our results show that in the cytosol, GPx activity seems to be less effective as H2O2 concentration increases probably due to an accumulation of GSSG, as GSH regeneration is slow in the RBC. GPx binding to the RBC membrane with increasing H2O2 is most likely an effort to protect the membrane as GPx can remove hydroperoxides, contributing to LPO prevention.
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119. The interactions between the metastasis suppressor, NDRG1, and the endoplasmic reticulum response in cancer cells

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The metastasis suppressor, N-myc downstream regulated gene-1 (NDRG1), is a stress response protein that is downregulated in cancers and this correlates with poorly differentiated, advanced cancers. It inhibits multiple oncogenic pathways and is upregulated by hypoxia, DNA damage or iron depletion. Initial studies have linked NDRG1 and the endoplasmic reticulum (ER) stress response. Considering this, we extensively examined the mechanism by which NDRG1 regulates the ER stress response in pancreatic and colon cancer cells.
The expression of NDRG1 was demonstrated to regulate the three main arms of the ER stress response by: (1) increasing the expression of three major ER chaperones, BiP, calreticulin, and calnexin; (2) suppressing the PERK pathway; (3) inhibiting the IRE1α arm; and (4) increasing ATF6 cleavage. An important finding was that NDRG1 enhances the anti-proliferative and anti-migratory activity of the anti-cancer chelator, Dp44mT. This increased efficacy could be related to the following effects in the presence of Dp44mT and NDRG1: activation of eIF2α; high cytosolic Ca^{+2} that increases apoptosis sensitivity; activation of CaMKII signaling; and increased pro-apoptotic CHOP. Collectively, this investigation dissects the mechanisms through which NDRG1 manipulates the ER stress response and its ability to potentiate the activity of the potent anti-cancer agent, Dp44mT.

120. Thiosemicarbazone-induced redox stress stimulates endoplasmic reticulum stress to inhibit cancer proliferation and migration

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The pro-survival pathways of the endoplasmic reticulum (ER), namely, the unfolded protein response (UPR), are activated in cancer and correlates with poor clinical outcomes. Moreover, this activation promotes malignant progression, chemoresistance and metastasis. An innovative strategy is to induce lethal pro-apoptotic activation of the UPR using novel thiosemicarbazones (TCs) that form cellular redox active metal complexes. TCs demonstrate potent anti-tumour/-metastatic activity *in*

4) tap water (in the drinking water). Animals were weighted daily and after 7 days were euthanised. Different bowel segments were isolated and occludin, claudin-5 as well as MPO and iNOS were analysed. Fecal bacterial DNA was studied by DGGE before and after treatment. Nitrate prevented antibiotic-induced body weight loss ($p<0.05$) and cecamegalia ($p<0.05$). Gastric expression of occludin and claudin tended to decrease during dysbiosis but both protein levels were recovered by nitrate ($p<0.05$). Also, nitrate inhibited MPO and iNOS overexpression upon antibiotherapy ($p<0.05$). Antibiotics eradicated most of gut flora ($p=0.0016$), reducing microbiota richness by 56%, while nitrate attenuated such microbial loss (48%, $p=0.068$). Hence, nitrate consumption may be recommended during antibiotherapy.

149. Linkage of cytosolic antioxidant enzymes to the erythrocyte membrane as protection mechanism in hereditary spherocytosis

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Our aim was to evaluate the binding of cytosolic antioxidant enzymes to the erythrocyte membrane and its relationship with well-known oxidative stress biomarkers - membrane bound hemoglobin (MBH) and membrane lipid peroxidation (LPO) - in Hereditary Spherocytosis (HS) patients.
We studied 74 HS patients, assessing MBH and LPO by spectrophotometry and the erythrocyte membrane bound amount of catalase (CAT), glutathione peroxidase (GPx) and peroxiredoxin 2 (Prx2), by western-blot.
CAT, CAT+GPx or CAT+GPx+Prx2 were detected in 43, 7 and 24 HS patients, respectively. The total amount of the three enzymes linked to the membrane was strongly correlated with MBH ($r = 0.497$, $p<0.001$) but not with LPO ($r = 0.156$, $p = 0.183$). We also evaluated the levels of MBH and LPO according to the quartiles of membrane bound enzymes amount and found that, while MBH significantly increased, LPO showed no changes between the different quartiles.
Our results show that, in HS, the linkage of antioxidant enzymes to erythrocyte membrane may provide a protection mechanism against the oxidative stress associated to this disease, as these enzymes are H2O2 and hydroperoxide scavengers and we observed that LPO seems to be contained.
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150. Anthocyanidins promotes beiging of white adipose tissue in mice fed a high fat diet

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Supplementation with anthocyanidins (AC), i.e. cyanidin and delphinidin, caused a decrease in body weight gain and fat deposits in mice fed a high fat diet (HF). This study investigated the capacity of dietary AC to promote "beiging" of subcutaneous white adipose tissue (sWAT), through increased mitochondrial biogenesis and thermogenesis. C57BL/6J male mice were fed control (C) or HF diets, with or without supplementation with 40 mg AC/kg body weight (CA and HFA). After 15 w, HF consumption caused obesity and increased eWAT weight, which were mitigated by AC supplementation. Adipocyte diameter was higher in HF than in C, CA and HFA mice. H&E stain showed images compatible with beiging in both AC-treated groups. Electron microscopy (TEM) showed a fewer number of mitochondria in HF but not in HFA mice. TEM findings were confirmed by measuring the expression of mitochondrial protein markers. AC prevented HF-induced inhibition of the pathway leading to mitochondria biogenesis (PPARγ, PRDM16, PGC-1α), and thermogenic respiration (UCP-1). Mice consuming the control diet plus AC also showed upregulation of PPARγ, PRDM16, PGC-1α. Findings suggest that consumption of selct AC could be an important strategy to mitigate HF-induced obesity in part via activation of adipocyte mitochondriogenesis and beiging.

151. Neuronal control of intestinal iron-homeostasis in the nematode *Caenorhabditis elegans*

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Oxygen and iron are essential for many fundamental processes of life including DNA and ATP synthesis. Nevertheless, dysregulated oxygen and iron can result in excessive formation of oxygen radicals that can cause tissue injury and death. Therefore, cellular oxygen and iron metabolism should be tightly regulated at all times. However, how this is achieved at the whole animal level is still mysterious. Here, we explore this question using the nematode *Caenorhabditis elegans*. We found that the conserved iron-cage protein ferritin 1 (*ftn-1*) is differentially expressed in the intestine during hypoxia and reoxygenation stress. Intriguingly, we show that *ftn-1* expression is controlled by oxygen -sensory neuron signalling. Furthermore, our data suggest that hydroxylated HIF-1 inhibits *ftn-1* expression, while non-hydroxylated HIF-1 has no function in *ftn-1* regulation. Finally, we demonstrate that *ftn-1* upregulation in hypoxia protects against *Pseudomonas aeruginosa* bacteria. Together, our studies provide novel insights into the way oxygen and iron responses are regulated at both the cellular and whole animal level.

152. (-)-epicatechin mitigates *in vivo* and *in vitro* high fat-induced apoptosis, oxidative and endoplasmic reticulum stress in pancreatic β-cells

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