dehydrogenase and GAPDH purified proteins, which are known to be glutathionylated, for *in vitro* experiments..This work shows active involvement of cytosolic Glo II for *in vitro* protein S-glutathionylation. To confirm the role of Glo II, preliminary protein-protein docking studies was performed between Glo II and human actin. The data showed a high propensity to aggregate with other proteins through its catalytic site Further, *in silico* investigation of Glo II stability and behavior, conducted through full atom molecular dynamics simulations, showed an high folding stability together with a great affinity towards its own reaction product glutathione both protonated (GSH) and unprotonated (GS^-). These studies, revealed that GloII, using its natural substrate SLG, allow a rapid and specific protein-SSG formation, leading enzymatic regulation of S-glutathionylation in proteins of different origin and cellular compartmentalization.

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P18

The effect of resveratrol on signal transduction pathways and the role of pro-apoptotic Bax protein on apoptosis in HCT-116 colon carcinoma cell lines

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Abstract

Colon carcinoma is the third among the cancer related deaths. The role of pro-apoptotic Bax protein on the resveratrol related apoptosis, mitochondrial membrane potential and signal pathways has not been identified in colon carcinoma cells. In this direction, HCT-116 bax positive and HCT-116 negative cell lines were utilized to detect the apoptotic effect and IκB, MEK1 and STAT 3 signal transduction pathways of resveratrol. The impact on the cell viability and IC50 value of resveratrol has been determined via WST-1 viability assay. The ratio of apoptosis has been evaluated via flow cytometry following Annexin V/Propidium iodide (PI) double staining. Changes in the mitochondrial membrane potential have been analyzed by flow cytometry and JC-1 fluorometric staining. IkB, MEK1 and STAT3 molecules were measured by Enspire device. Data showed the IC50 value for resveratrol as 50 μM. According to the flow cytometry, apoptosis ratio has been determined as 29.65% in the experimental group of bax positive cells, as 13.98% in the experimental group of bax negative cells. Changes in the membrane potential has been established as 8.62% in the experimental group of bax positive cells, as 97.98% in the experimental group of bax negative cells. When the obtained data from Enspire device was reviewed; bax positive cells IκB phosphorylations were found as as 5.22 for experimental groups; MEK1 phosphorylations were found as and as 1.15 for experimental groups; STAT 3 phosphorylations were found as and as 2.52 for experimental groups. In HCT-116 bax negative cells, IκB phosphorylation were and 2.71 in experimental groups; MEK1 phosphorylation were 1.18 in experimental groups; STAT 3 phosphorylation were 1.54 in experimental groups. Our data show that Bax protein plays role in the apoptotic effect of resveratrol by altering mitochondrial membrane potential and mitochondrial membrane permeability, signal transduction and the absence of Bax increase the sensitivity of HCT-116 colon carcinoma cells to apoptosis.

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P19

Quantification of Carnosine-Aldehyde Adducts in Human Urine

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Abstract

Lipid peroxidation generates several reactive carbonyl species, including 4-hydroxy-2-nonenal (HNE), acrolein (ACR), 4-hydroxy-2-hexenal (HHE) and malondialdehyde. One major pathwayof aldehydes detoxification is through conjugation with glutathione catalyzed by glutathione-S-transferases or, alternatively, by conjugation with endogenous histidine containing dipeptides, such as carnosine (CAR). In this study, on-line reverse-phase high-performance liquid chromatography (HPLC) separation with tandem mass spectrometry detection was utilized for the accurate quantification of CAR- ACR, CAR-HHE and CAR-HNE adducts in human urinary samples from non-smokers young adults. Standard adducts were prepared and isolated by HPLC. The results showed the presence of a new product from the reaction of CAR with ACR. This new adduct was completely characterized by HPLC/MS-MSn, 1 H RMN, COSY and HSQC. The new HPLC/MS/MS methodology employing stable isotope-labeled internal standards (CAR-HHEd5 and CAR-HNEd11) was developed for adducts quantification. This methodology permits quantification of 10 pmol CAR-HHE and 1 pmol of CAR-ACR and CAR-HNE. Accurate determinations in human urine sample were performed and showed 4.65 ± 1.71 to CAR-ACR, 5.13 ± 1.76 to CAR-HHE and 5.99 ± 3.19 nmol/mg creatinine to CAR-HNE. Our results indicate that carnosine pathways can be an important detoxification route of α, β -unsaturated aldehydes. Moreover, carnosine adducts may be useful as redox stress indicator.

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P20

Disruption of the iron-sulfur cluster of aconitase by myeloperoxidase-derived oxidants

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Abstract

Oxidative damage catalysed by the heme enzyme myeloperoxidase (MPO) has been linked with multiple inflammatory pathologies. The major oxidant species generated by MPO are hypochlorous (HOCl) and hypothiocyanous acids (HOSCN). The damage induced by HOCl is well characterized and has been linked to multiple diseases, however the role of HOSCN is less well understood. It is known that HOSCN can cause selective damage, as this oxidant selectively targets thiol (e.g. Cys) residues and selenium-containing species. The aim of the current study was to assess whether HOCl and HOSCN can disrupt the [4Fe-4 S] cluster of aconitase causing iron release and loss of activity.

It is shown that HOSCN induces rapid and efficient release of iron from aconitase, with 80% removed at an oxidant concentration of 3 micromoles/mg protein; this is markedly more efficient than HOCl. In contrast the extent of loss of enzymatic activity was comparable between the two oxidants at the same concentration. Blocking the [4Fe-4 S] cluster inhibited HOSCN-mediated inactivation, but did not have dramatic effects on HOCl-mediated damage, consistent with HOSCN, but not HOCl, interacting with the cluster. This data is supported by peptide mass mapping studies

that indicate that HOSCN oxidises Cys385 of the [4Fe-4 S] cluster. In contrast HOCl damaged multiple sites.

Exposure of human coronary artery endothelial cells (HCAEC) to 0-50 micromolar HOCl or 0-150 micromolar HOSCN resulted in an increase in intracellular iron, loss of aconitase activity and a loss of mitochondrial aconitase protein. In contrast cytosolic aconitase was not affected.

These data indicate that aconitase - and particularly the mitochondrial form - is a target for MPO-mediated damage with HOSCN showing a selectivity for the [4Fe-4 S] cluster and inducing greater iron release. This damage, and the release of iron, may exacerbate oxidative stress in cells at sites of inflammation where active MPO is present.

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P21

Hypercholesterolemia as a risk factor for depressive disorder?

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Abstract

Aims: Epidemiological findings demonstrated that increased plasma cholesterol levels are frequently observed in depressive patients. In this regard, there is enhancing evidence that hypercholesterolemia is associated with impairment of brain function. Recently, we demonstrated that low-density lipoprotein receptor knockout ($LDLr^{-/-}$) mice- a widely used rodent model of familial hypercholesterolemia - exhibited memory deficits and cortico-cerebral mitochondrial dysfunction. In this study, we aimed to assess the hypercholesterolemic mice in predictive tasks for depressive-like behavior.

Methods: Adult wild type C57BL/6 and $LDLr^{-/-}$ mice were evaluated in two tests for depressive like behavior, the splash test and forced swimming test. In addition, the activity of monoamine oxidase isoforms and the mRNA levels of hemeoxygenase-1 were assessed in the hippocampus and cerebral cortex of C57BL/6 and $LDLr^{-/-}$ mice. Finally, the blood-brain-barrier (BBB) permeability was investigated using the AQP-4 immunofluorescence staining in the mice hippocampus.

Results: The $LDLr^{-/-}$ mice showed a significant reduction in the grooming time in the splash test and increased immobility time in the forced swimming test, and both parameters were reversed by fluoxetine antidepressant treatment (10 mg/kg, 7 days, o.g.). Interestingly, the depressive like behavior of $LDLr^{-/-}$ mice was associated with increased activity of monoamine oxidase A, decreased hemeoxygenase-1 mRNA levels and increase of BBB permeability in the hippocampus.

Conclusions: Overall, these data provide new evidence that hypercholesterolemia could trigger brain alterations involved in depressive disorders.

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P22

De novo ceramides synthesis is not involved in skeletal muscle atrophy induced by short-term mechanical unloading

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Abstract

Patients admitted to the intensive care unit commonly develop skeletal muscle weakness that can exacerbate illness and complicate their recovery. Beyond the primary disease or aging, weakness is promoted by a variety of prolonged hospitalization-associated conditions. These include altered nutritional status, pharmacologic side effects, physical inactivity, and prolonged bed rest. The two latter conditions (i.e. inactivity and bed rest) are the most ubiquitous, affecting all patients during a prolonged hospitalization. In both cases, skeletal muscle utilization is decreased with a concomitant reduction in fatty acid oxidation. Subsequent fatty acids accumulation converted to ceramides could be a cellular mechanism leading to muscle wasting. Indeed these sphingolipids act as second messengers in several of molecular signaling pathways involved in muscle atrophy. Consequently, the aim of this work is to determine the effects of immobilization on muscle ceramides accumulation, and identify the role of these ectopic lipids in molecular mechanisms involved in skeletal muscle atrophy. For this purpose, male Wistar rats were treated with an inhibitor of de novo synthesis of ceramides (i.e. myriocin) and subjected to hindlimb unloading for 7 days. We found that hindlimb unloading induced skeletal muscle atrophy, in part through proteolysis (i.e. decrease in AKT activation, increase in MuRF1 and polyubiquinated proteins content) and apoptosis activations (i.e. increase in Bax/Bcl-2 ratio and cleaved caspase-3). Myriocin treatment did not prevent skeletal muscle atrophy and concomitant induction of apoptosis and proteolysis. Data concerning muscle ceramides content are being analyzed. Together, these results suggest that de novo synthesis of ceramides is not involved in muscle atrophy induced by a short period of hindlimb unloading.

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P23

Singlet molecular oxygen generated in dark biological process

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Abstract

Ultraweak chemiluminescence arising from biomolecules oxidation has been attributed to the radiative deactivation of singlet molecular oxygen [$^aO_2$] and electronically excited triplet carbonyl products involving dioxetane intermediates. As examples, we will discuss the generation of $^aO_2$ from lipid hydroperoxides, which involves a cyclic mechanism from a linear tetraoxide intermediate. The generation of $^aO_2$ in aqueous solution via energy transfer from the excited triplet acetone arising from the thermodecomposition of dioxetane a chemical source, and horseradish peroxidase-catalyzed oxidation of 2-methylpropanal, as an enzymatic source, will also be discussed. The approach used to unequivocally demonstrate the generation of $^aO_2$ in these reactions is the use of ^{18}O-labeled hydroperoxide / triplet dioxygen ($^{18}[^cO_2]$), the detection of labeled compounds by HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) and the direct spectroscopic detection and characterization of $^aO_2$ light emission. Characteristic light emission at 1,270 nm, corresponding to the singlet delta state monomolecular decay was observed. Using $^{18}[^cO_2]$,