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Abstracts

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Complex I and other big complexes in mitochondria

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Complex I (NADH:ubiquinone oxidoreductase), the most complicated and largest component of the mitochondrial respiratory chain, is involved in neurodegenerative disorders and plays a pivotal role in the generation of reactive oxygen species (ROS). Using redox proteomics we could define specific targets that are oxidized depending specifically on the ROS source within the mitochondrial respiratory chain [1].

Despite its central importance our knowledge about complex I is still rather limited, mainly since until recently insufficient structural information was available at the atomic level. We have solved the structure of complete mitochondrial complex I from *Yarrowia lipolytica* to a resolution of about 3.9 Å resolution [2,3]. Structural and functional studies reveal that the distal domain of the membrane arm indeed pumps half of the protons across the bioenergetic membrane [4]. In combination with extensive mutagenesis studies the structural data show that the ubiquinone reduction site resides a remarkable ~30 Å above the membrane near the interface between the 49-kDa and the PSST subunit of the peripheral arm. We have explored the entry pathway for ubiquinone leading to a conserved tyrosine next to iron-sulfur cluster N2 [5]. Using short chain ubiquinone analogues with site-directed mutations, we could identify a hydrophobic gate for the hydrophobic tail of the substrate [6]. A comparison of the structure of the mitochondrial enzyme to the recently published structure of bacterial complex I form *Thermus thermophilus* reveals remarkable differences some of which can be interpreted in terms of the active/deactive transition of complex I involving a possible redox switch mechanism.

In our long standing quest to study large macromolecular assemblies in mitochondria, we developed Complexome Profiling as bottom up approach to study the inventory of multiprotein complexes in a given sample [7]. This approach not only allowed us to identify TMEM126B as component of the Mitochondrial Complex I Assembly factor (MCIA) complex, but also led to the discovery of a giant complex transiently formed during Ca^{2+}-induced mitochondrial Permeabilty Transition (mPT). This 30 MDa mPT-complex consists of more than 100 different proteins and seems to be formed by recruiting smaller mitochondrial complexes that normally execute various other functions [8]. The presence of essentially all proteins previously implicated in this process including the nucleotide carrier, VDAC and cyclophilin D suggests that the observed mPT complex could indeed serve as the long searched-for mitochondrial permeability transition pore.

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Three-dimensional structure of mitochondria in the living cells

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It is well established that mitochondria in the living cells are not simply granular, but filamentous and continuously divide and fuse. Namely, the morphology of mitochondria results from the equilibrium between two opposing processes, fusion and fission. In the present talk, I would like to discuss about two problems which remain to be solved.

1. Biogenesis of mitochondria in mammalian cells during the cell cycle progression:

It is well established that in yeast cells, mitochondria fuse together making one huge mitochondrion, and it is divided into two. One part of mitochondrion is transported to a daughter cell using actin cable. On the other hand, in mammalian cells, it is generally accepted that each mitochondrion in the cell divides into two just before the cell divide, and two daughter cells contain an equal number of mitochondria. Previously, we proposed a new model for the biogenesis of mitochondria (1): taxol- and nocodazole-treated cells are arrested in G_2/M phase, and mitochondria get together around aggregated chromatin of the nucleus. Mitochondria were assembled as a spherical structure in taxol- or nocodazole-treated cells that are three-dimensionally reconstructed by their confocal microscopic images. Namely, it is possible in mammalian cells a similar phenomenon may occur to the case of yeast cells, described above. We need further investigation.

2. The correlation between megamitochondria(MG) formation induced by free radicals (2) and stress-induced mitochondrial superfusion (SIMH) discovered by Tondera et al. (3):

When cells are subjected to modest levels of stress (UV irradiations, actinomycin D), mitochondria hyperfuse and form a highly interconnected network. At least four dynamin-related GTPases, mitofusin 1(MFN1), mitofusin 2 (MFN2), optic atrophy (OPA1), and dynamin-related protein 1(DRP1) are known to play essential role in fusion and fission. SIMH was found to be independent of MFN2, BAX/BAK, and prohibitins, but requires L-OPA1, MFN1 and the mitochondrial inner membrane protein SLP-2. Tondera et al. found that intracellular level of ATP is significantly elevated in cells undergoing SIMH. They claim that the role of SIMH is for the maintenance of mitochondrial ATP production under stress. On the other hand, ATP synthesis in MG is generally lowered. The rate of the generation free radicals from MG is lowered. Thus, we proposed that MG formation is an adaptive process to oxidative stress. Gene analysis on free radical-induced MG is lacking at the moment, and analysis on molecular mechanism of MG formation is essential to solve the correlation with SIMH.

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Do the uncoupling proteins protect mitochondria against oxidative stress? Proposal for a novel mechanism

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Uncoupling protein-1 (UCP1, thermogenin) was identified some 35 years ago in mitochondria of the brown adipose tissue. Its thermogenic function is well established. It produces leakiness of the inner mitochondrial membrane to protons, thus dissipating the mitochondrial electrochemical proton gradient and producing heat. During last 15 years homologues of UCP1, or of their mRNAs, have been found in numerous mammalian tissues, like heart, skeletal muscles, thymus, lungs, brain, etc. and designated as UCP2, UCP3, UCP4 and UCP5. Analogues of mammalian UCPs have also been detected in birds, fish, insects, plants, fungi and protists. Although they all are classified as uncoupling proteins (UCPs), their precise function and importance are debated. Mild uncoupling, equivalent to lowering of the mitochondrial electrochemical proton gradient, has been proposed to decrease the rate of generation of oxygen reactive species (ROS). Other suggested mechanisms include export of the excess of fatty acids from mitochondria to the cytosol and translocation of fatty acid peroxides and lipid hydroperoxides. We have proposed [1,2] that UCP3 in rat heart and skeletal muscle mitochondria and UCP2 in mouse brain mitochondria can function as transporters of the superoxide anion, thus contributing to the extrusion of this noxious oxygen free radical from the mitochondrial inner compartment to the intermembrane space and further on to the cytosol. We found that high ROS release to the incubation medium by mitochondria from rat heart and skeletal muscles and from mouse brain was substantially decreased by purine nucleoside di- and tri-phosphates GDP and GTP, known to inhibit UCPs. Among two components of ROS that were determined in the incubation medium, hydrogen peroxide and superoxide anion radical, only the latter was decreased by GDP or GTP. In contrast, intramitochondrial level of ROS, as assessed by inactivation of the matrix enzyme aconitase, was increased by GDP and GTP. These effects of GDP and GTP on the release of ROS were not observed or were much less expressed in mitochondria of brains isolated from transgenic mice deprived of UCP2.

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Aluminum-induced oxidative changes in different blood cells: an “in vitro study”

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Aluminum (Al) is a redox-inert element thought to induce cell damage via activation of oxidative stress. In this work, the effect of aluminum on different cellular compartments of both, human erythrocytes and peripheral blood lymphocytes was studied.

The presence of aluminum induced lipid peroxidation at the membrane level.

Physico-chemical modifications were studied using different fluorescent probes able to localize in various parts of the phospholipid bilayer (TMA-DPH, laurdan and pyrene).

A decrease in fluorescent anisotropy of TMA-DPH and in the polarity of the lipid bilayer with a concomitant shift toward a gel phase was observed while the pyrene excimerization coefficient (kex) increased.

The presence of aluminum reduced the activity of erythrocyte antioxidant enzymes (SOD, CAT and GSHPx) and induced morphological changes on the erythrocyte membrane surface as monitored using atomic force microscopy.

Flow cytometry measurements were performed in lymphocytes to evaluate the effect of $AlCl_3$ on the intracellular production of ROS and mitochondrial membrane potential using carboxy-H2-DCFA and Rhodamine123, respectively.

Flow cytometry results indicate that Al induced reactive oxygen species generation and mitochondrial membrane depolarization.

Finally, we explored lymphocytes DNA susceptibility to damage using “comet assay” (single-cell gel electrophoresis). Aluminum induced only slight nuclear DNA damage.

These results provide further information on the target of action of different concentrations of aluminum.

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Biological impact of perhydrolysis

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Prevention of oxidative protein modifications

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Oxidative protein modifications, adversely affecting protein functions, accumulate with aging, are augmented in various diseases and contribute to their pathogenesis. E. g., protein carbonylation is elevated in a plethora of diseases involving oxidative stress. Advanced glycoxidation and lipoxidation end products (AGEs and ALEs) contribute to the development of not only diabetic complications, but also of other pathologies. It can be expected that prevention/removal of posttranslational protein modifications may ameliorate the development of various diseases and contribute to life extension and healthy aging. Different strategies have been employed for this purpose, as exemplified for AGEs and ALEs: prevention, inhibition and removal. Antioxidants and metal chelators are useful for prevention of oxidative stress, which generates or facilitates AGEs and ALEs formation. Carbonyl quenchers i. e. compounds trapping reactive carbonyl derivatives, both non-enzymatic and enzymatic, are also effective on the prevention stage. The second level of intervention consists in acceleration of the catabolism of already formed AGEs/ALEs and includes both potentiation of activities of endogenous proteolytic systems and using xenobiotics able to catalytically degrade AGEs/ALEs. The third level of intervention concerns blocking the biological response to AGEs/ALEs, mediated by receptors to these substances (RAGEs), and covers the use of antagonists of such receptors and inhibition of signaling pathways initiated by activation of RAGEs. Natural compounds effective at any level of intervention can be employed as nutraceuticals; new synthetic compounds can become useful drugs.

Some Aspects of Nitric Oxide and its Metabolites in Biology

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Nitric oxide ($^{\bullet}$NO) is one of the smallest and simplest biologically active molecule in nature and one of the most ubiquitous substances in mammalian species. It has important beneficial effects, such as vasodilation, but, at the same time, it may became toxic through its metabolites.

In these last decades, $^{\bullet}$NO has been object of interest for a number of researchers, both from the biological and the chemical point of view.

Nitric oxide is, itself, rather unreactive towards most of the biological molecules: it reacts rapidly only with oxygen (k=10^6 M^{-1} s^{-1}) producing nitrogen dioxide and nitrous anhydride, or with superoxide anion (k is diffusion controlled) affording peroxynitrite. These metabolites are good hydrogen abstractors, nitrating agents and oxidants and are responsible for nitrating and oxidative stress in biological systems.

In the present communication, some aspects concerning the detection of nitric oxide (1,2) and the deleterious effects of nitrogen dioxide will be discussed (3.4)

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Oxidative stress, aging and inflamm-aging - A study on mitochondrial DNA

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Oxidative stress has been considered a primary driver of the ageing process, but recently this tenet has been put in discussion, as in animal models the abrogation of oxidative stress did not alter life span, while an increased oxidative burden results in unaltered or even increased life span. In humans, clinical trials with antioxidants have often failed. These results lead to conclude that either these studies are misleading, and oxidative damage is, in fact, a major determinant of ageing, or that molecular damage caused by ROS is not a major primary cause of aging; i.e., the oxidative stress theory of ageing is untrue. We recently proposed that a possible link between oxidative stress and ageing can be inflammation, as it is known that oxidative stress elicits inflammation though multiple mechanisms, and that ageing is characterized by increased levels of circulating pro-inflammatory molecules such as CRP and IL-6. We proposed to name this status of chronic, low level of inflammation as "inflamm-ageing". A brief overview of the most recent developments of the inflamm-ageing theory will be given.

It is known that mitochondria are the primary source of reactive oxygen species (ROS) and that they are the sole organelles that possess their own DNA (mtDNA). MtDNA variability is likely affecting in organelle' functionality as well as ROS production, therefore it is expected that, if mitochondria are important for ageing, a different distribution of mtDNA inherited or somatic variability should be present among long living subjects and younger controls. In past studies, mtDNA haplogroup J resulted to be associated with longevity but was also found to predispose to Leber's Hereditary Optic Neuropathy (LHON). To reappraise the correlation between mtDNA variability and longevity, mtDNAs from samples of more than 2200 nonagenarians (and an equal number of controls) collected within the framework of the GEHA EU project were categorized by high resolution classification and by complete sequencing. A complex, unexpected scenario emerged where mutations in subunits of OXPHOS complex I had a beneficial effect on longevity, while the simultaneous presence of mutations in complex I and III (which also occurs in J subhaplogroups involved in LHON) was found to be detrimental. Moreover, mutations in tRNA genes, previously associated to degenerative disorders, were found to be correlated with longevity. On the whole, our data suggest that mtDNA variation does affect human longevity, but its effect is heavily influenced by the interaction between mutations concomitantly occurring on different mtDNA genes and by the interaction with nuclear variability. Further studies on mtDNA heteroplasmy have revealed that also the proneness to accumulate mtDNA mutations is under genetic control, as it resulted to be similar in parent-offspring couples, as assessed by deep sequencing in 30 centenarians, their offspring and 27 individuals whose parents died prematurely age- and sex-matched with centenarian' offspring.

Age-related changes of mitochondrial functions in human fibroblasts

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Considerable evidence indicate that aging affects mitochondrial structure and function, although the severity of dysfunctions is different among human individuals and cells types. Most biochemical investigations have shown a decrease of the mitochondrial oxidative phosphorylation (OXPHOS) during aging (Lenaz G. et al. 2006), but other studies linking mitochondrial functions and longevity have given conflicting results. In addition, autophagy, that plays a main role in mitochondrial homeostasis, was found to be impaired in aging suggesting that it can contribute to the age-related accumulation of respiratory defects in tissues, but the mechanisms underlying this process in long living individuals (LLI) are being poorly investigated.

The aim of the present study was to evaluate the possible relationship between the mitochondrial function and ageing in LLI. In particular, we focus on the possible contribution of the autophagic mechanisms to preserving the cellular energetic competence during prolonged lifespan as in LLI. The study was carried out using as experimental model human fibroblasts taken from three cohorts of different aged individuals.

To determine whether the respiration rate and the oxidative phosphorylation efficiency were affected by aging, both the ATP synthesis rate and the ATP level were assayed (Sgarbi G. et al. 2006). Complex I driven ATP synthesis rate showed a significant reduction in fibroblasts mitochondria of LLI compared to both young and old donors, whilst complex II driven ATP synthesis was only scarcely affected. Nonetheless, fibroblasts of LLI showed significantly higher ATP levels. Interestingly analysis of the mitochondrial mass showed an age-dependent increase, resulting significantly more marked in LLI. Moreover analysis of the supramolecular organization of the OXPHOS complexes showed a higher content of the ATP synthase dimer in LLI fibroblasts compared to young donors.

Interestingly, fluorescence microscopy analysis of the mitochondrial network showed elongated mitochondria that resemble the network morphology of cells exposed to nutrient starvation, condition proposed to protect mitochondria from mitophagy and to sustain cell viability (Gomes et al. 2011; Rambold et al. 2011; Scherz-Shouva et al. 2007)).

Taken together the data showed that fibroblasts obtained from LLI present a decline in mitochondrial function but this alteration is compensated by an increase of mitochondrial mass, that in turn could be the result of protective mechanisms against mitophagy.

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Complex III dysfunction and superoxide production: a bacterial versus human comparative case study

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Complex III (ubiquinol:cytochrome c oxidoreductase; CIII) is a multisubunit membrane bound enzyme that is central for respiratory energy transduction pathways. In its native form CIII is a symmetrical homodimer and, depending on the species, it contains up to eleven subunits. The catalytic core of complex III is formed by three universally conserved subunits from bacteria to mammals, namely cytochrome b, iron-sulfur protein and cytochrome c1. Cytochrome *b* is the only mtDNA-encoded subunit of the mitochondrial CIII and provides the quinone pockets that operate according to the Q-cycle hypothesis. It is generally accepted that the major production of superoxide anion in CIII is at the quinone binding site of cytochrome b (Q_o), where quinol oxidation occurs. Studies using bacterial CIII highlighted the importance of the structural integrity of Q_o site for maximal rate of catalysis and minimal electron leakage to oxygen, identifying some specific aminoacid residues as key contributors for affecting ROS production. In particular, it has been recently shown that the conserved tyrosine residue at position 302 of *Rhodobacter capsulatus* cytochrome *b* is critical for protection against oxidative damage of CIII. In fact, its substitution with other amino acids and in particular cysteine, decreases CIII activity and concomitantly enhances superoxide production. Interestingly, the same mutation at position 278 of human cytochrome *b* (p.278Y>C, m.15579A>G) has also been encountered in a patient with severe exercise intolerance and a multi-system disorder. Here, we describe and compare the properties of the bacterial mutant enzyme with its mitochondrial counterpart. Similarities between the bacterial and human mutant CIII are remarkable, including decreased catalytic activity of CIII and enhanced ROS production. This case illustrates the usefulness of undertaking parallel and complementary studies using biologically different yet evolutionarily related systems. It also deepens our understanding of the mechanism of CIII function and ROS production, and highlights the importance of supramolecular organization of bacterial and mitochondrial respiratory chains (i.e., respirasomes) and their potential disease associated protective roles.

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Inactivation of protein tyrosine phosphatases by oxidizing compounds

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Protein tyrosine phosphatases (PTPs) dephosphorylate proteins at phosphotyrosine residues, and together with protein tyrosine kinases are responsible for the regulation of tyrosine phosphorylation status controlling numerous cellular processes.

The hallmark defining the classical PTP enzymes is the strictly conserved active site sequence $C(X)_5R$ within the catalytic domain which constitutes the phosphate-binding pocket of the enzyme. The cysteine residue located at the bottom of the active site cleft exists in the thiolate anion form and is highly susceptible to oxidation. Oxidation of cysteine residue leads to the formation of a reversible form of sulfenic acid residue, while highly oxidizing environment can induce further oxidation yielding physiologically irreversible sulfinic and sulfonic acid residues, all of which consequently cause inactivation of the enzyme.

In the present work, the effects of hydrogen peroxide and highly oxidizing peracids have been studied. Peracids can be produced as a result of the reaction between carboxylic acids and hydrogen peroxide. The peroxycarboxyl group (O=C-O-OH) in peracid is an oxidized derivative of the regular carboxyl group (O=C-OH). Peracids can undergo decomposition, forming carboxylic acids, and releasing singlet oxygen.

The performed experiments demonstrate that nanomolar concentrations of peracids can inactivate recombinant protein tyrosine phosphatase CD45 as well as CD45 natively present in a Jurkat cell line. Moreover, the experimental results show that compared to hydrogen peroxide, peracids have higher inhibitory effect on PTP CD45. The experiments demonstrate that carboxylic acids have no impact on PTP CD45 activity, which indicates that the peroxycarboxyl group rather than nonspecific impact of hydrocarbon chain is directly implicated in the mechanism of enzymatic inhibition.

According to the computational molecular dynamics modelling, peracids (especially medium-chain) are predicted to have stronger binding affinities than hydrogen peroxide for the PTP CD45 active site. Estimates of the binding enthalpy for selected residues in the CD45 catalytic center show that C8-peracid may interact with histidine residue, which is able to react with singlet oxygen.

The structural comparison reveals only minimal changes in the enzyme conformation in the presence of peracids, suggesting that the structural changes are not likely to influence the inactivation of CD45.

The obtained data allow us to assume that peroxycarboxyl group of peracids may be directly implicated in the mechanism of protein tyrosine phosphatase CD45 inhibition. Moreover, the experimental data suggest that inactivation of PTP CD45 by peracids is caused by the oxidation of the cysteine residue in the catalytic center, rather than by steric hindrance due to strong ligand binding in the catalytic center.

Differentiation of epidermis modulates reactive oxygen response in the skin

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The epidermis of the skin forms a natural barrier protecting organism homeostasis from outer and inner stress signals. It is composed mainly of keratinocytes cells which undergo continuous regeneration in order to maintain its function. These cells' growth and differentiation result in formation of distinct epidermal layers of cells on different stages of development with unique structure and function.

The aim of the study is to examine how the differentiation of keratinocytes alternates skin stress response system. Neuropeptides such as corticotropin releasing hormone are molecules that play central role in systemic response to stress but it is also established that skin cells express its equivalent. We have shown that besides the characteristic changes in expression of keratinocyte differentiation genes, neuropetides and their receptors undergo upregulation, suggesting alternation of stress response pathways during differentiation process. Besides the central regulatory functions of neurohormonal axis, skin also directly can response to particular stressors, such as reactive oxidative and nitrosative species (ROS and RNS) generated for instance after UV irradiation of the skin.

In this study it was shown that calcium - the main regulator of skin differentiation – regulates expression of the gene involved in ROS and NOS response. It seems that more differentiated keratinocytes are more protected against high doses of H_2O_2. Moreover, the affect of calcium is also time dependent and induction of differentiation decreases immediate response (4 h) but strengthen long-term reaction (24 h) in studied keratinocyte population.

In conclusion, process of differentiation of keratinocytes is accompanied by increased level of stress response both on the neurohormonal level and direct ROS and NOS response.

Impact of cell culturing conditions on expression of nitrosooxidative stress-induced genes in cellular model of osteosarcoma

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INTRODUCTION: Osteogenesis apparent during fracture healing or during distraction osteogenesis (bone lengthening or bone correction) is a time-taking physiological process. Various pathological situations like infection, disturbed distraction, bone dysplasiae, non-/delayed union (being both a cause as well as a result of lengthy treatment) create a vicious circle of delayed consolidation. Tissue engineering has been recently employed in order to enhance osteogenesis in troublesome clinical situations.

Experimental tissue engineering technique necessitates 3 important factors: cells, scaffold, and signals. Thus success of in-vitro bone formation relies on cells (osteoblasts, osteoblast progenitors or stem cells), signals for osteoblastic differentiation (extracellular agents or interaction between cells) and proliferation (growth factors) as well as an optimal culture environment. Unfortunately cells cultured in vitro in a traditional monolayer cultures are susceptible to damage not only from mechanical processing, but also from stress reactions (deviations from optimal growth conditions that inhibit or disturb cell growth). Oxidative stress provokes, at best, a cellular adaptive response. Reactive Oxygen Species (ROS) involve in biological phenomena, such as mutation, carcinogenesis, aging, and inflammation. DNA strand breakage, inappropriate cross-linking of proteins and lipid oxidation occurs.

These processes in ex-vivo cultures may have direct practical implications for clinical application of bioengineered tissues.

MATERIALS AND METHODS:

Osteosarcoma cells were cultured in monolayers, collagen and Matrigel. Cultures will be dissolved at time intervals and examined for cell viability and oxidative and nitrosative stress markers. Changes in expression of oxidative and nitrosative stress will be evaluated, compared between groups and outcomes discussed.

STUDY AIMS:

To investigate the effect of a novel 3D osteosarcoma culture model on oxidative stress

(improved intercellular signaling and possible reduction of oxidative stress).

RESULTS:

Three dimensional environment modifies oxidative stress, its influence on gene

expression, proliferation, death and potential risks to cultured cells. This environment better protects cultured cells from oxidative stress, which poses possibilities for safer and more efficient bone engineering.

Mitochondrial respiratory supercomplexes limit ROS production

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The mitochondrial respiratory chain is known today to be organized in supramolecular assemblies that are called supercomplexes [1]. Besides conferring a kinetic advantage due to channelling of mobile substrates (ubiquinone and cytochorome c) and being required for the assembly and stability of Complex I, indirect considerations support the view that supercomplexes may also prevent excessive formation of reactive oxygen species (ROS) from the respiratory chain, in particular from Complex I (CI) and Complex III (CIII). It has been demonstrated that such supramolecular organization can be affected by the metabolic state of the cell (plasticity model, cf. [2]) and that aging is also accompanied by a decline of supercomplex content [3]. Those studies, however, failed to clearly show which is the causing event (i.e. whether supercomplex dissociation is a cause or a consequence) because concurrent phenomena were observed.

In this perspective, our study was performed in proteoliposomes containing CI and CIII from bovine heart mitochondria; when the proteoliposomes were built at a phospholipid/protein ratio of 1:1, CI and CIII were associated in the form of the supercomplex I_1III_2 (SC), as shown by BN-PAGE. Treatment of the proteoliposomes with detergent (dodecyl maltoside) was able to dissociate the SC into the individual complexes; alternatively, SC was not formed when the proteoliposomes were prepared at high phospholipid/protein ratio (30:1). We provide experimental evidence that a dramatic decrease of NADH-cytochrome c reductase activity occurred when the SC was dissociated, while the activity of CI was not altered, showing that channelling of Coenzyme Q_{10} in the SC had been shifted to the less efficient “pool behaviour”. Preliminary results also indicate that reconstitution of the proteoliposomes at 30:1 PL/protein ratio in the presence of 20% cardiolipin restore Coenzyme Q_{10} channelling as shown by the enhancement of NADH-cytochrome c reductase activity.

In all cases when the SC was dissociated or not formed, a significant increase of ROS formation from CI was detected as an enhancement of the fluorescence of the probe dichlorofluorescein.

This is the first demonstration that dissociation of SC is a cause of oxidative stress from CI that may perpetuate a vicious cycle of ROS generation and bioenergetic impairment. It is easy to foresee the implication of these findings for human diseases and aging.

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3-hydroxytyrosol, signal transduction and microRNAs in chondrocytes exposed to oxidative stress

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Chondrocyte death and extracellular matrix degradation represent important components in cartilage degeneration and the mode of cell death may play a critical role in the pathogenesis of osteoarthritis (OA) [1].

Recent findings attributed a potential role to autophagy in the regulation of the cellular response to several stress stimuli. Although its role is context and tissue-dependent and still unclear, autophagy has been observed to decrease during aging and several age-related diseases, including OA. Thus, it is not weird that some studies indicate the "longevity factor" Sirtuin-1, a NAD-dependent deacetylase, as a potent inducer of autophagy [2].

In the last decades several scientific investigations have been performed to discover aliment or food constituents that provide health and medical benefits ("nutraceuticals"), which might be useful for prevention or treatment of chronic degenerative- and aging-associated diseases [3].

Therefore we have tested the ability of 3-hydroxytyrosol (HT) to protect chondrocytes from cell death in vitro. HT is a natural phenolic compound primarily released in olive mill wastewater and in olive oil.

We have found that HT pre-treatment of growing cultures of C-28/I2 chondrocytes (an immortalized human cell line) and primary cultures of human chondrocytes (prepared from fragments of articular cartilage obtained from adult OA patients) reduces cell death, inflammation and matrix degradation markers induced by oxidative stress (H_2O_2). In particular our results indicate that HT prevents matrix metalloproteinase-13 (MMP-13) and cyclooxygenase-2 (COX-2) increases. The protective effect seems to be mediated by decreased phosphorilation of c-Jun N-terminal kinase (JNK), which results elevated following treatment with H_2O_2.

Preliminary results indicate that HT causes a SIRT-1 increase and, at the same time, a microRNA-9 decrease. This data suggest that HT might regulate SIRT1 expression at post-transcriptional level, maybe through the modulation of the microRNAs network, which has recently emerged as an important cellular regulator. Moreover, on the basis of some data, we hypothesize that the beneficial effect of HT might be mediated by an involvement of mechanisms of autophagy.

Controlled inhibition of key steps in this process could represent a novel therapeutic strategy for OA treatment and cure. Furthermore it is already accepted and recognized the clinical potential of microRNAs in several diseases, whereas it is much less known about the role of these small RNAs in the pathophysiology of OA as well as of other age-related diseases.

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NO2* in ALS, when the good turns bad

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Nitrooxidative stress was found to take part in Amyotropic Lateral Sclerosis (ALS) and other neuroinflammatory diseases. Beside multiple, not fully known yet, genetical factors leading to the disease, common activation of proinflammatory cytokines leads to phagocytic activation of microglia. Those supportive and immune cells were found to be main source of reactive oxygen and nitrogen species during infection-like injury. Nitroperoxidation of cell membranes components and proapoptotic switch of NGF function were found significant for nitrogen reactive species induced neuronal death. Inhibition of prooxidative enzymes seems to be beneficial for survival in experimental pathologies.

Using spinal cord tissue from hSOD-1 G93A transgenic rats on different level of disease progression with direct method of *NO2 measurement based on *cis*-$[Cr(C_2O_4)(AaraNH_2)(OH_2)_2]^+$ we managed to show significant increase of the radical in affected tissue. ($AaraNH_2$) = methyl 3-amino-2,3-dideoxy-α-d-*arabino*-hexopyranoside. The results indicate on importance of reactive nitrogen species, particularly NO2*, in neurodegenerative diseases.

Alzheimer's disease, oxidative stress and impact of gender

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There are many evidences suggesting that oxidative stress is one of the earliest events in Alzheimer disease (AD) pathogenesis and plays a key role in the development of the AD pathology. In addition, growing bodies of evidence indicate that the etiopathogenetic mechanisms of AD differ significantly depending on gender.

The present work was conducted in order to investigate the role played by nitric oxide (NO) and peroxynitrite (ONOO-) in platelets from AD patients both male (M-AD) and female (F-AD), the intracellular Ca2+ concentration ([Ca2+]i) and the membrane Na+/K+-ATPase activity and fluidity. NO production was significantly elevated in platelets from both F-AD and M-AD compared to matched controls. M-AD showed NO production significantly higher than F-AD and it was the same between M and F controls. A similar trend was seen for ONOO-. Platelets of both M-AD and F-AD had intracellular Ca2+ concentrations significantly higher than control, while membrane Na+/K+-ATPase activity showed an opposite trend, but these differences are still significant. M-AD subjects showed a significantly increased DPH fluorescence anisotropy compared with controls, while for F-AD this discrepancy was not significant. The difference in DHP fluorescence anisotropy remained significant between M-AD and F-AD as well as between M and F controls. The TMADPH fluorescence anisotropy showed the same trend, but there were no significant differences between M-AD and F-AD, as well as between controls.

In conclusion, the increased expression and activity of nitrergic system may produce platelet membrane alteration or vice versa. These modifications may contribute further to the neurodegenerative process in AD because the abnormal function of Alzheimer platelets plays a very important role in the pathogenesis of the disease. Moreover, these results support the conclusion that F-AD is not at greater risk than M-AD for oxidative stress injuries. Studies on gender differences could lead to a higher probability of improved health outcomes via better-targeted therapies.

CoQ_{10} and R,S-alpha lipoic acid prevent oxidant mediated mitochondrial dysfunction without affecting intracellular ROS levels. A new role as mitohormetic nutrients?

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Reactive Oxygen Species have long been considered a necessary by-products of oxidative metabolism. Mitochondria, in this respect, represent a target of oxidative damage and their impairment might lead both to decreased energy production and enhanced ROS formation. However, free radicals not only cause damage but also have a physiological role in cell signaling, underlying adaptation to stress and training. A tight control of redox status is therefore critical in preserving pivotal cellular functions. This concept is summarized in the mitohormesis hypothesis that outlines how mild oxidative stress physiologically, produced by mitochondria, is endowed with ergogenic effects and promotes metabolic health. While severe oxidative damage underlies different pathologic conditions, the use of antioxidants such as vitamin C has been shown to hamper physiologic ROS mediated adaptive processes. The aim of the present study was to investigate whether supplementation of mitochondrial nutrients with antioxidant activity was able to prevent oxidant-mediated mitochondrial dysfunction without abolishing intracellular ROS levels associated with adaptive mechanisms. Investigation was conducted ex-vivo, in Peripheral Blood Lymphocytes (PBL) collected from 16 healthy subjects before and after supplementation, for two weeks with 200 mg Coenzyme Q10 alone or in association with 200 mg Lipoic Acid. Intracellular ROS levels, mitochondrial functionality and DNA damage was evaluated in control PBL and in cells challenged with an oxidative insult. Data have shown that CoQ10 supplementation produced a significant protection of mitochondrial functionality even in the strong oxidising environment applied. However, intracellular ROS levels and transitory DNA damage were not decreased by antioxidant treatment.

Effect of oxidative stress on HNE metabolism

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Oxidative stress arises from an imbalanced redox status between the production of reactive oxygen species (ROS) and the biological systems able to remove them. A common biochemical aspect in cancer cells is the generation of ROS able to induce lipid peroxidation phenomena.

The most intensively studied end product of lipid peroxidation is 4-hydroxy-2,3-nonenal (HNE) (Esterbauer H. et al., 1991).

It is known that in carcinogenesis, increased ROS level can inhibit tumor cell growth and high concentrations of HNE can induce apoptosis in cancer cells. Indeed, several anticancer drugs and radiation therapy, that increase oxidative stress, can overcome the antioxidant defences of cancer cells and drive them to apoptosis. However recent advances shown an important and physiological role of HNE in cell signalling (Robino G. et al. 2001). In addiction main attention was given to cellular detoxification of HNE and to the possible role of the aldehyde as signaling molecule during oxidative stress in association with important diseases such atherosclerosis and cancer.

This research is focused on the study of the pattern of HNE-metabolizing enzymes and on the modulation of their activity during oxidative stress in order to control the intracellular level of the aldehyde. A characterization of the enzymatic activities, with a great attention on HNE metabolizing enzymes (i.e. aldose reductase, glutathione S-transferase and aldehyde dehydrogenase) were performed in human astrocytoma ADF cells under physiological and oxidative stress conditions. A different degree of severity of oxidative stress was imposed by incubating the cells in the presence of hydrogen peroxide and HNE. At the end of incubation cell viability, metabolites content and HNE metabolizing enzymes activities were evaluated. While no changes in aldose reductase and glutathione S-transferase occurred, a significant thiol-dependent reduction of NAD^+ dehydrogenases activities were detected. Hydrogen peroxide treatment causes a reduction of NAD^+ content and an increased level of oxidized glutathione, mainly as GS-proteins mixed disulphides, which appears proportional to the severity of the oxidative insult.

Hydrogen peroxide enhances effects of Vitamin D on human keratinocytes

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Vitamin D exerts pleiotropic effect that is more sophisticated than the traditional involvement in regulation of calcium homeostasis. The noncalcemic role of vitamin D includes its direct and indirect engagement in the cell cycle and proliferation, differentiation, apoptosis as well as immune properties.

It is already known that vitamin D analogs inhibit proliferation of many cell types, including human keratinocytes, through the cell cycle arrest. Another key role in growth inhibition is induction of apoptosis by vitamin D and its anologues.

We have confirmed the inhibitory effects of 1,25-dihydroksyvitamin D3 on the cell cycle in HaCaT cell line, in concentration dependent manner, as well as the induction of apoptosis. Moreover, effect exerted by calcitriol was substantially enhanced in the presence of 1 milimolar concentration of hydrogen peroxide.

According to our observations the cell cycle arrest caused by 1,25-dihydroksyvitamin D3 was prominently noticed during the G2/M transition together with growing fraction of apoptotic cells held in the subG1 fraction. Still this effect was emphasized in the presence of hydrogen peroxide.

Expression of classical genes activated by reactive oxygen species, such as catalase, SOD1, SOD2, was not influenced by 1,25-dihydroksyvitamin D3 alone. It suggest involvement of some another genes or possibly nongenomic interactions between vitamin D3 and hydrogen peroxide.

Synergistic effect of 1,25-dihydroksyvitamin D3 and hydrogen peroxide on cell proliferation suggest potential use of oxidative drugs together with vitamin D3 in the treatment of hyperproliferative diseases.

The toxicity of co-exposure to fluoride and silver nanoparticles: mechanisms and clinical significance

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According to the World Health Organization the most common worldwide oral diseases are dental caries and periodontal diseases affecting 60–90% of world population. Fluoride treatment is considered to be crucial for protection against these diseases. However, an excess of fluoride is also associated with systemic toxic effects causing *fluorosis*. Fluorosis may result from occupational exposure or from excessive intake of fluoride in drinking water and food. Currently, growing use of nanoparticles has also been of concern and led to the rise of nanotoxicology.

Therefore I have studied both toxicological outcomes and mechanisms of co-exposure to fluoride and silver nanoparticles, which are often used in various dental preparations i.e tooth paste and mouth wash. We have found that co-exposure to both xenobiotics led to enhanced cell and organ damage and these effects are associated with generation of oxidants leading to inflammatory reactions and apoptosis. Furthermore, our studies point to significant interactions between fluoride and silver nanoparticles leading to generation of reactive oxygen species, decrease in total antioxidant status, stimulation of lipid peroxidation and gingival fibroblast death. Thus, co-exposure of these cells to fluoride and silver nanoparticles increases cytotoxicity.

The significance of all these environmental threats and interactions for population toxicology remains to be investigated.

Diallyl trisulfide-induced iron-dependent reactive oxygen species formation is mediated by JNK1-ITCH-p66shc signalling axis in prostate cancer cells

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Prooxidative activity of thiol-reactive cyclopentenones in signaling pathways leading to autoelimination of osteosarcoma

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Bone cancer constitutes approx. 6% neoplasms of the childhood. Osteosarcoma accounts for up to 20% of all the bone cancer cases diagnosed in children with the annual incidence of 5 per 1 million population under the age of 20 years. Combined neoadjuvant and adjuvant therapy increases 5-year survival rate from 10-20% up to 40-60%. There has been no improvement in the survival rate since 1970, when the combined chemotherapy was introduced. It has been discovered that prostaglandins of J series of (PGJ) regulate processes like inflammation and tumorigenesis, many a time inducing programmed cell death in cancer cell lines that are specifically resistant to apoptosis. The mechanism of PGJ-induced cell death mode leading ultimately to necrosis is not known, however, it has been associated with ER stress, and disturbance of protein sulfhydryl homeostasis caused by a thiol-reactive cyclopentenone ring structure.

The aim of the project was to investigate the effects of 15d-PGJ2, PGJ2, as well as other selected natural and synthetic 2-cyclopenten-1-one derivatives on highly metastatic human osteosarcoma 143B cell line, focusing specifically on antiproliferative properties and oxidative stress parameters in the signaling pathway leading to autoelimination of the cancer cells.

The osteosarcoma 143B cell line was obtained from the American Tissue Type Collection. Cells were cultured under standard conditions and treated with various concentrations of 15d-PGJ2, PGJ2, PGA2, PGE2 and cyclopentenone. Inhibtion of cell growth was investigated by MTT assay. Induction of apoptosis and reactive oxygen species production were determined by flow cytometry analyses.

Prostaglandins 15d-PGJ2, PGJ2 and PGA2 (all being cyclopentenone derivatives) inhibited cell growth and proliferation of osteosarcoma 143B cells with the estimated EC50 of 10.7 µM, 10.8 µM and 13.9 µM, respectively. Prostaglandin PGE2 (being a cyclopentanone derivative) did not significantly affect cellular growth and proliferation. Interestingly, synthetic cyclopentenone alone did not affect osteosarcoma 143B cells growth either. Moreover, prostaglandins 15d-PGJ2, PGJ2, PGA2, and to a lesser extent PGE2 as well as cyclopentenone, increased the level of oxidative stress. However, only 15d-PGJ2 and PGJ2 were observed to markedly induce both apoptosis and necrosis in osteosarcoma 143 cell line. The results suggest that the mechanism of PGJ2-induced programmed cell death of osteosarcoma 143B cells is not solely dependent on the cyclopentenone ring structure, and oxidative stress phenomena seem to be one of the signaling pathways potentially involved in the autoelimination of cancer cells. The obtained results may be of clinical importance, as an increased necrotic response of osteosarcoma to effective chemotherapy dramatically improves prognosis.

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Mitochondrial dysfunction implicates ROS-independent antitumorigenic effects via HIF1α destabilization

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The recently defined function of *oncojanus* concerns the ability of high loads of mutations in NADH-ubiquinone-oxidoreductase (complex I) mitochondrial DNA-encoded genes to arrest tumor growth by impeding hypoxia inducible factor-1α (HIF1α) stabilization and, hence, adaptation to hypoxia. In this frame, we also showed that the complex I dysfunction that follows the occurrence of disruptive mutations of the mitochondria-coded subunits does not implicate an increase in reactive oxygen species, which in turn does not impact on the pro- or anti-tumorigenic properties of the oncojanus. Through allotopic expression of wild-type MTND1 oncojanus in MTND1-null osteosarcoma cells, we managed to recover complex I assembly and NADH-oxidase activity, shifting the balance of Krebs cycle metabolites α-ketoglutarate/succinate, essential to recuperate HIF1α stabilization. Allotopic cells injected in mice generated larger and more aggressive tumor masses compared to MTND1-null cells. Next-generation-sequencing whole-transcriptome profiling and metabolites measurement of allotopic versus MTND1-null xenografts showed that, despite remaining prevalently glycolytic, NADH-oxidase activity was fundamental to activate survival pathways needed for tumors progression and adaptation to hypoxia. Our study defines a specific role in tumorigenesis of a fundamental metabolic enzyme such as the multiprotein respiratory complex I and identifies its NADH-oxidase function as a potential anti-cancer target.

Melatonin and Vitamin D - two small molecules with big potentials

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Melatonin and vitamin D are the evolutionarily ancient molecules, what suggests that their activity in the living organisms go far beyond classic, endocrine targets. Melatonin, in an addition to light-dependent regulation of circadian and reproductive cycles is also involved in free radical scavenging, DNA protection and repair, as well as body weight control, coat pigmentation and immunomodulation. Vitamin D, on the other hand is not only well established regulator of calcium homeostasis, but also is essential modulator of cell proliferation and immune response. Such as diverse functions are mediated through high-affinity receptors or through direct interactions with multiple crucial proteins. Both molecules, as well as, their metabolites and analogs, were shown to possess receptor-independent activities including proapoptotic, antiproliferative and antioxidative properties. Moreover, production and metabolism of melatonin and vitamin D is no longer restricted to classical locations (pineal gland for melatonin and skin-liver-kidney metabolic pathway for vitamin D) as it was proven that many peripheral organs including skin are not only targets but impotent, local source of those hormones.

In the skin cells, those molecules play important role in response to stressors such as ultraviolet irradiation on multiple levels including direct scavenging of reactive oxidative/nitrosative species (it is well documented for melatonin so far) or indirectly thought their receptors: expressional regulators, protein cofactors and immunomodulators.

Interestingly, besides different mechanisms of action their beneficial effects may be concentration-dependent, because high doses of melatonin or vitamin D were shown to inhibit proliferation of several cancer derived cells including melanomas. Thus, it seems that melatonin and vitamin D play important role as an intra-, auto- para and endocrine regulator of local (e.g. skin) as well global homeostasis.

Tracking the structural changes of a protein during its misfolding on atomic level

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Protein folding and unfolding are crucial for a range of biological phenomena and human diseases. Defining the structural properties of the involved transient species is therefore of prime interest. Using a combination of cold-denaturation with nuclear magnetic resonance spectroscopy we reveal detailed insight into the unfolding of the homodimeric repressor protein CylR2. Seven three-dimensional structures of CylR2 at temperatures from 25 °C to -16 °C reveal a progressive dissociation of the dimeric protein into a native-like monomeric intermediate followed by transition into a highly dynamic, partially folded state. The core of the partially folded state appears critical for biological function and misfolding [1].

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Free radicals in acute pancreatitis

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Immune-cell Therapy of the Advanced Pancreatic Cancer

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Since cancer cells are transformed from normal cells via a series of mutations during a long time, they express mutated proteins that are antigenic as 'non-self'. Therefore, cancer cells might be attacked and rejected by the immune system. In order to develop a new method of cancer therapy, a number of trials are going on using immunology; peptide vaccines, monoclonal antibodies, and immune cells.

Here I present our trials on the advanced pancreatic cancer using own immune-cells, e.g., ex-vivo expanded T-lymphocytes (CD3–LAK) and antigen peptide-charged dendritic cells (DC). In CD3-LAK, T-Lymphocytes were expanded 500-fold ($5x10^9$) by culturing in vitro for 2-week in the presence of anti-CD3 monoclonal antibody and IL-2. DC ($4x10^7$) was differentiated from monocytes in the presence of IL-4, GM-CSF, and TNF-α, then charged with peptide antigens such as MUC-1.

Among 85 pancreatic cancer patients of stage IV, 67 were treated with LAK every 2 week, 5 were treated with LAK every week, and 13 were treated with DC+LAK every 2 week. Median survival time from diagnosis (MST) was 18- month in the DC-LAK group, which is significantly longer than that in LAK-2W group (14-month). In LAK-1W group, MST was 16-month. These MST were much longer than the chemotherapy alone: 8.8-month with gemcitabine, 9.7-month with S1 (GEST test, 2012, Japan). Therefore, the immune-cell therapy, especially, that with DC, is effective for extending MST of advanced pancreatic cancer.

With respect to other cancers, it has been shown that the immune-cell therapy is also effective for improving MST in cancers of stomach, colon, and lung, in their advanced stages.

An interaction between 2-methoxyestradiol and geldanamycin in osteosarcoma cell death model

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Understanding of the regulatory mechanisms of carcinogenesis and drug-induced apoptosis seems to be a major milestone on the way to develop effective and safe anticancer therapies. Osteosarcoma (OS) is one of the most malignant bone tumors of childhood and adolescence. In light of many studies, 2-methoxyestradiol (2-ME) and geldanamycin (GA) could become potent and relatively safe agents for treatment of OS patients.

The aim of the study was to determine and compare the anticancer effects of either 2-ME or GA, or a combination of both on the osteosarcoma cell lines.

To our studies we employed highly metastatic OS 143B and MG63.2 cell lines. Neuronal nitric oxide synthase (nNOS), heat shock proteins (HSPs) gene expression and protein level were determined by means of Real Time PCR, Western blotting and immunofluorescence.

We proved that both 2-ME and GA inhibit cell growth and induce cell death of OS cells, however they are more effective when used alone. One of the possible mechanisms of negative interaction and lack of synergistic effects of 2-ME and GA seem to be their contradictory impact on nNOS, and major HSPs.

Oxidative stress and high density lipoproteins (HDL) in health and disease

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High density lipoprotein (HDL) plasma levels are inversely correlated with the risk of atherosclerosis and coronary heart disease (CHD). The best known function of HDL is the capacity to promote cellular cholesterol efflux from peripheral cells and deliver cholesterol to the liver for excretion, thereby playing a key role in reverse cholesterol transport (RCT). The functions of HDL that have recently attracted attention include anti-inflammatory and anti-oxidant activities. High antioxidant and anti-inflammatory activities of HDL are associated with protection from CHD.

The atheroprotective properties of HDLs originate from their unique composition and structure. HDLs are highly complex particles and thousands of different proteins and different lipids are associated with HDLs. Along with this complexity, a multitude of potent interferences with the structure and function of HDLs have been described and linked with diverse disease states, in particular cardiovascular high risk diseases such as obesity, kidney disease and diabetes.

In these pathological conditions HDLs undergo several modifications in structure and composition mediated by various mechanisms including oxidation, glycation, homocysteinylation or enzymatic degradation. Both *in vitro* and *in vivo* studies have demonstrated that these compositional alterations affect HDL functional and atheroprotective properties. Either oxidized HDL or glycated HDL progressively lose their normal biological activities and acquire altered properties and are converted into a 'dysfunctional' HDL. Dysfunctional HDL are characterized by decreased levels and activities of anti-inflammatory and anti-oxidant factors, such paraoxonase -1 (PON1) and could became pro-inflammatory particles. Therefore, dysfunctional HDL could contribute to accelerated atherosclerosis in degenerative diseases.

Oxidative stress and complicated pregnancy

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INTRODUCTION

An aging-suppressor gene, klotho, is a candidate factor for vascular disease because its deficiency leads to impaired endothelium-dependent vasodilation and impaired angiogenesis.

OBJECTIVES

The aim was to verify a possible relation among the expression of the klotho gene, single nucleotide polymorphisms (SNP) in the promoter region, and placenta aging.

METHODS

Placentas were collected from normal pregnancies (n= 34) and pregnancies complicated by Preeclampsia (n= 34), matched for gestational age. Klotho mRNA and protein were determined using Real-time quantitative PCR and Western blot, respectively.

SNPs (i.e.: -744delA, and -395A/G) were investigated using allele-specific PCR. Expression of pluripotency markers (i.e.: Nanog, and Oct-4) and telomere length measurement were assessed using Real-time PCR.

RESULTS

Real-time PCR analyses demonstrated a significant down-regulation of Klotho (83 %; p = 0.005) in patients with Preeclampsia versus Controls. Results of Western Blot agreed with Real-Time PCR ones. Polymorphism analysis results suggest that -744delA allele is associated with 3-fold increased risk for preeclampsia. Real-time PCR investigation revealed a significant down-regulation of pluripotency markers in pathological group.

CONCLUSIONS

Klotho expression is decreased in preeclamptic pregnancies. Further data are required to confirm the role of this protein in pathophysiology of preeclampsia and the possible link to long term outcomes.

Activity of glutathione reductase in podocytes is associated with activation of polyol pathway

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Podocytes, highly differentiated cells in kidney glomeruli, play a crucial role in maintaining glomerular filtration barrier. Due to inability to replicate, their damage and loss leads to irreversible changes in the kidney. Hyperglycemia activates polyol pathway (PP) of glucose metabolism , which belongs to major pathomechanisms of diabetic complications. Increased activity of aldose reductase (AR), a key enzyme of PP can be associated with massive depletion of intracellular NADPH pool, a cofactor that is also used by glutathione reductase (GR) for the reduction of oxidized glutathione (GSSG) to GSH. The competition for NADPH could be responsible for chronic oxidative stress in podocytes leading to their impairment in diabetic kidney.

Aim: The aim of our study was to examine whether increased activity of AR in podocytes cultured in diabetic conditions is associated with alterations in activity of GR.

Methods: Immortalized mouse podocytes were cultured in RPMI 1640 with different glucose content and osmolarlity: (A)-NG-Nosm,normal (5,5mM) glucose and normal (280 mOsm) osmolarity, (B)-NG-Hosm, normal glucose and high (380mOsm) osmolarity, and (C)- HG-Hosm, high glucose and high osmolarity. The cells were incubated in these media for the period from 6 hours to 5 days. Enzyme activities were determined spectrophotometrically, as the rate of conversion of NADPH to NADP at 340 nm. All results were compared to NG-Nosm group in respective time points.

Results:

High osmolarity alone stimulated AR activity (by 82%±0.004 , $P<0.05$ vs control) after 6-hour incubation in NG-Hosm medium. A drop in GR activity was observed after 24 hours only. At the same time, activity of AR returned to control level. After 5 days, AR activity remained unchanged while GR activity increased substantially. 6-hour exposure to high glucose and osmolarity (HG-Hosm) moderately increased AR activity , while GR activity strongly increased. After 24 hours, no changes in AR and tiny drop in GR activities were observed . A 5-day incubation in HG-Hosm resulted in substantially increase of GR activity.

Conclusion: Glucose- and osmolarity-induced changes in activities of AR and GR are not parallel. Strong activation of AR is followed by a drop in GR activity which was observed 18 hours later. Similarly, decrease of AR activity is followed by increased activity of GR. It seems likely that in diabetic podocytes, disturbances in the intracellular redox state due to inhibition of GR activity may be driven by upregulation of polyol pathway enzyme, AR.

Physical activity and chronic diseases of old age

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Aging is a multifactorial process resulting in damage of molecules, cells, and tissues. These damages predispose older adults to progressive weakening , chronic disabling disease, and mortality. Frailty can be defined as a state of vulnerability that carries an increased risk of adverse health outcomes in older persons. Identification of frail persons is of interest for both research and clinical practice. The physical phenotype of frailty, derived by Fried et al from the Cardiovascular Health Study (CHS) cohort, defines frailty on the basis of five features mainly related to sarcopenia. Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength. The European Working Group on Sarcopenia in older people recommends using the presence of both low muscle mass and low muscle function (strength or performance) for the diagnosis of sarcopenia. The combination of sarcopenia and obesity is frequent in older persons and further increases the risk of mobility disability and functional impairment.

Several mechanisms may be involved in the onset and progression of sarcopenia. In many older people, the aetiology is often multifactorial. Possible causes include aging itself, sedentary lifestyle, advanced organ failure, inflammation, hormonal changes, and malnutrition. Oxidative stress has been suggested to be responsible for some of these causes. Oxidative stress represents an imbalance between production of reactive oxygen species (ROS) and antioxidant cellular systems unable to cope with the excess of oxidants. ROS are continuously formed during life as a result of oxygen metabolism, and their production is increased during some pathological processes.

Exercise is an ideal intervention to counteract the effects of aging and oxidative stress on muscle.

Physical activity increases the expression and the activity of antioxidant enzymes, with consequent reduction of ROS. In particular, there is growing evidence that physical activity, along with several other longevity-promoting interventions, may actually cause an activation of mitochondrial oxygen consumption and promote increased ROS formation. However, ROS serve as molecular signals exerting downstream effects which ultimately induce endogenous defense mechanisms culminating in increased stress resistance.

Antioxidant effects depend on exercise protocol. Recommendable physical activity should be aerobic, with constant and regular exercise training, not vigorous or intense, and tailored to the characteristics of the subjects in order to avoid injuries. Interventions combining exercise with caloric restriction appear particularly effective for counteracting sarcopenic obesity.

Challenges in Alzheimer's disease drug discovery

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Alzheimer's disease (AD) is still an incurable disease, which causes cognitive decline, irreversible memory loss, disorientation, language impairment, ultimately leading to death. The currently available drugs for the treatment of AD are just symptomatic, representing three inhibitors of the acetylcholinesterase enzyme (i.e. donepezil, rivastigmine, and galantamine), and memantine, a non-competitive NMDA receptor antagonist. The difficulty for developing a satisfactory therapy of AD lies in the complex pathophysiology of the disease, which involves the alteration of numerous pathways, whose temporal occurrence is still poorly understood. Known alterations include deficiency in cholinergic neurotransmission, defective β-amyloid protein metabolism, accumulation of aggregated Tau proteins within neuronal cells and involvement of inflammatory, oxidative and hormonal pathways, also accompanied by neurovascular dysfunction. Thanks to the growing knowledge on the molecular mechanisms, several AD drug candidates, hitting the specific targets involved in the amyloid plaque formation, tau protein hyperphosphorylation and oxidative stress, have reached the last phases of clinical trials and new drug discovery avenues have been pre-clinically investigated to offer a more effective treatment. In this talk these potential new drugs will be presented, along with the discussion of their therapeutic features and limits.

Molecular bases of protein aggregation related to neurodegenerative diseases

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Protein misfolding very often leads to the serious pathogenic consequences, like Alzheimer and Parkinson diseases that are nowadays the two most frequent neurodegenerative disorders. Proteins involved in neurodegeneration processes are found to be globular structured proteins, like parvulins, which are known to be associated with protein folding [1] and intristically dissordered proline-rich polypeptides. Parvulins are known for their Peptidyl-Prolyl Isomerase activity (PPIases) that catalyze the *cis – trans* rotation around the Xaa-Pro peptide bonds in target proteins/peptides. The intrinsically disorder proteins, like Tau protein and amyloid precursor protein (APP) were found to form insoluble aggregates *in vivo* in the absence of PPIases [2]. It is worth mentioning that proteins, which undergo the pathological aggregation process leading to neurodegenerative diseases are characterized by high content of Thr-Pro/Ser-Pro motifs within their sequences. Some data suggest that the polyproline II region of conformational space is a key conformation in IDPs and important for pathogenic aggregation [3].

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Neonatal permethrin exposure induces long-term neurodegeneration: role of DNA methylation

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Early life environmental exposure to pesticides could play a critical role on the onset in adulthood of some age-related diseases [1-2], and it is the main cause of epigenetic pattern alterations that vary from tissue to tissue and change with aging [3].

Previous studies on rats exposed to low doses of permethrin from 6th to 21st day of life, have shown many adverse effects, in adulthood such as the central and peripheral impairment of redox and immune systems, with a significant increase in plasma pro-inflammatory cytokines [4,5]. Besides, early life exposure to permethrin induces neurodegeneration in adulthood of the striatum characterized by a decrease in Nurr1 gene and protein expression, lower dopamine levels, accelerated dopamine turnover, protein and lipid oxidation together with decreased GSH levels [6,7].

Nurr1, the gene responsible for the development and maintenance of dopaminergic system, was analyzed considering an epigenetic approach devoted to clarify the mechanisms associated with its down-regulation.

Acknowledgements

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Comparative biology of genotoxic stress resistance

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Although different species are made by the same biochemical building blocks (i.e. DNA, proteins, lipids and carbohydrates) their longevity varies widely (for example maximum longevity is 4 years for a mouse and probably more than 210 years for a whale). In addition considering that the amount of damaging insults species are receiving from the environment is similar per unit of time and that the observed differences in internal ROS production do not match the observed difference in longevity. Our hypothesis is that a large contribution to longevity must derive from a differential capacity to handle damage.

We have chosen mammalian primary skin fibroblast lines that can be easily kept and challenged with damaging agents under identical conditions, focusing our attention to one of the most profound kind of damage that cell can encounter: DNA double strand breaks (DSBs). We have found that the capacity to recognize damaged DNA (measured as DNA-end binding activity) increases exponentially with longevity[(1)], and correlate with the abundance of the first proteins involved in DNA damage recognition Ku80, Ku70 and DNA PK. These proteins are necessary for Non Homologous End Joining (NHEJ); others protein such as rad51 (important for Homologue Recombination, HR) and ATM (important to activate the DNA damage response) do not appear to be more expressed in long lived species.

We have used γH2AX and 53BP1 foci formation as markers of DNA damage. Surprisingly, according to foci counts, human DNA should be considered more fragile than mouse DNA, in fact, after the same exposure to damaging agents such neocarzinostatin or etopocide we measured higher number of foci in human fibroblasts compared with mouse ones. Using a more definitive marker of DNA damage, i.e. micronuclei formation (that can derived either from DSBs or mitotic spindle malfunctioning) it appears, instead, that foci formation, more than representing a direct measure of the endured damage, represents the cells “awareness” of the endured damage[(2)]. Cells more “aware” of the endured damage such as human cells seems to be more efficient in slowing or stopping cells cycle progression. Our data indicate that the S and/or the G2 check points seems to be more fundamental for longevity than the G1[(2)] or the mitotic spindle check point[(3)]. These findings have prompted us to propose an integration to the disposable soma theory of ageing. We propose that a key element for a long lived species is, more than having cells with large proliferative capacity[(4)], having cells that can take their time to repair the endured damage and to make accurate choices regarding if to keep proliferating or enter senescence or apoptosis[(5)].

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Recent developments in the biomedical and clinical aspects of Coenzyme Q_{10}

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The use of CoQ_{10} in cardiovascular disease was originally based on the bioenergetic role of CoQ_{10} at mitochondrial level; more recently research ahs been focused on the involvement of CoQ_{10} in endothelial function. Following the initial observations of Gerald Watts in Australia, our lab has been deeply involved in studying the effect of CoQ_{10} administration, in patients affected by coronary heart disease (CHD), on endothelial function measured by the flow-mediated dilation (FMD) technique. In the same category of patients CoQ_{10}, at a dose of 300 mg/day, was also able to increase extracellular SOD (ecSOD) (Belardinelli et al. 2006, Tiano et al. 2007). A strong correlation was found between the increased plasma levels of CoQ_{10}, following its administration, and the improvement in FMD and in ecSOD activity. These vascular effects of CoQ_{10} may arguably be related to its key role in oxidative phosphorylation, which enables it to restore impaired mitochondrial function but it could also depend on its well-known antioxidant properties. A SOD-mimic activity of CoQ_{10} was highlighted by Greci et al. in 2000; this could subtract superoxide anion from reacting with nitric oxide, thus preventing its inactivation towards peroxynitrite. In recent years the anti-inflammatory and anti-apoptotic properties of CoQ_{10} have been investigated; these mechanisms could have important consequences at endothelial level. The latest observations from our group (Olivieri et al. FRBM in press) concern the ability of CoQ_{10}, in its reduced form, to affect gene expression by acting on the inflammatory pathways. A rapid increase in miR-146a expression has been reported during inflammatory and immune responses. IRAK-1 and TNF receptor-associated factor-6 (TRAF-6), established as molecular targets of miR-146a, are known to participate in the common signalling pathway affecting NF-κB-controlled gene expression. Thus, increased miR-146a expression during inflammatory responses might be involved in a negative feedback loop aimed to curb production of pro-inflammatory cytokines.

This biomarker combination including miR-146a, its target protein IRAK-1 and related interleukin-6 were collectively designated as MIRAKIL; activation of this pathway was found to indicate senescence-associated secretory phenotype (SASP) by primary human umbilical vein endothelial cells (HUVEC). We explored the ability of short and long term $CoQ_{10}H_2$ supplementation to affect MIRAKIL in HUVEC used as a model of vascular ageing; this effect was tested also in the presence of lipopolysaccharide (LPS), a known pro-inflammatory stimulus. LPS-induced NF-κB activation significantly decreased after $CoQ_{10}H_2$ pre-treatment both in young and senescent cells. The effect was more evident in young cells; in fact in senescent cells $CoQ_{10}H_2$ supplementation significantly attenuated LPS-induced miR-146a and IRAK-1 modulation, although it failed to curb IL-6 release. These CoQ_{10} properties may play a role in protecting endothelial function, possibly also in preventing endothelial dysfunction related not only with ischemic heart disease but also with major age-related disorders. Besides the clinical effects related to "therapeutic" plasma levels of CoQ_{10}, i.e. the increased blood levels that can be reached upon exogenous administration, the endogenous plasma CoQ_{10} levels have also been indicated to be a sensitive mortality-predictor in chronic heart failure (Molyneux et al. 2008). A recent study by Alehagen et al. (2012) highlighted a positive effect of CoQ_{10} and selenium formulation in improving survival in an elderly population.

Reduced coenzyme Q10 (ubiquinol) supplementation activates mitochondrial functions and decelerates senescence in senescence-accelerated SAMP1 mice

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Our recent studies have revealed significantly delayed senescence in Senescence-accelerated mouse prone-1 (SAMP1) mice given supplementation with reduced form of coenzyme Q10 (ubiquinol), but the mechanism of action of this effect has remained unclear (Yan J et al. *Exp Gerontol* 2006; Schmelzer C et al, *Mol Nutr Food Res*. 2010). Here, we report that dietary supplementation with ubiquinol prevents the age-related decrease in expression of Sirtuin1 and Sirtuin 3 (NAD^+-dependent protein deacetylases), and PGC-1α (a co-activator that controls mitochondrial biogenesis and respiration), and Ppar–α (peroxisome proliferator-activated receptor α) in the liver of SAMP1 mice. Protein levels of these genes and *Sod2* (superoxide dismutase 2), determined with western blotting analyses were also increased by supplementation. Supplementation with ubiquinol induced deacetylation of PGC-1α, SOD2 and IDH2 (Isocitrate dehydrogenase [NADP] mitochondriaation) proteins. Ubiquinol supplementation prevented age-related decrease in mitochondria complex I activity and increased numbers of mitochondria. Further, we found increased levels of phosphorylated CREB (cAMP response element binding protein). Activation of mitochondrial function by the induction of Sirtuin genes and PGC-1α may protect against the progression of aging and the symptoms of age-related diseases in SAMP1 mice **(Fig.1)**.

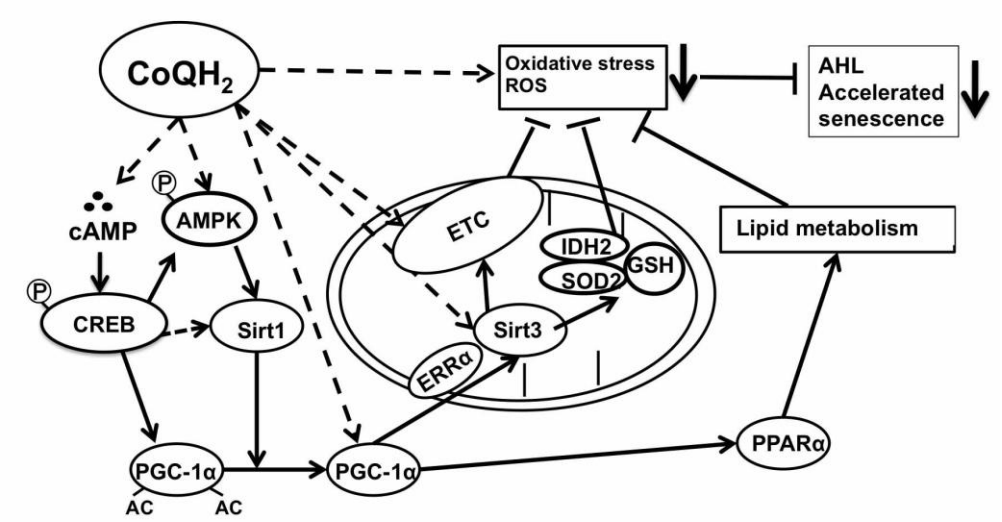

Fig.1 Mechanism of Anti-aging effects of $CoQH_2$

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Effect of a water soluble CoQ_{10} formulation on mitochondrial bioenergetics and oxidative stress

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Mitochondria are both the cellular powerhouse and the major source of reactive oxygen species. Coenzyme Q_{10} plays a key role in mitochondrial energy production and is recognized as a powerful antioxidant. For these reasons it can be argued that higher mitochondrial ubiquinone levels may enhance the energy state and protect from oxidative stress.

The rationale for CoQ10 therapy is supported by the evidence of decreasing CoQ10 levels with age in human and animal tissues, further suggesting a potential therapeutic role in age-related neurodegenerative disorders.

Despite these potential beneficial effects, clinical studies showed controversial results that are mainly due to the high hydrophobicity of this compound, which reduces its bioavailability. For our experiments *in vitro* and *in vivo* we used a water soluble CoQ_{10} formulation (Q_{ter}), obtained by terclatration of native CoQ_{10}. (1)

Initially we measured the cellular and mitochondrial ubiquinone content in two cell lines (T67 and H9c2) after supplementation with Qter and native CoQ_{10}. Our results show that the water soluble formulation is more efficient in increasing intracellular and mitochondrial ubiquinone levels. Then, we have evaluated the bioenergetics effect of ubiquinone treatment, demonstrating that CoQ10 content after Qter supplementation positively correlates with an improved mitochondrial functionality (increased oxygen consumption rate, transmembrane potential, ATP synthesis) and resistance to oxidative stress (2).

Considering the promising results from our *in vitro* studies, we exploited the beneficial effects of Qter treatment in a rat model of repeated noise exposure. This study addressed the relationship between cochlear oxidative damage and auditory cortical injury. We demonstrated that the systemic administration of Qter reduced oxidative-induced cochlear damage, hearing loss, and cortical dendritic injury by reducing the noise-induced redox imbalance in the cochlea and the deafferentation effects upstream the acoustic pathway (3).

Taken together these data represent a strong rationale for the clinical use of Coenzyme Q_{10} and highlights the enhanced biological effects of Qter both *in vitro* and *in vivo* studies.

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Reproductive ageing: antioxidants in the follicular microenvironment and the role of Coenzyme Q10

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Oxidative stress and accumulation of oxidative damage have been indicated as a major cause of age-associated infertility as well as other causes of reproductive impairment such as obesity, diabetes. The microenvironment of the follicle is vital for normal oocyte development, folliculogenesis, and timely regulated ovulation.

Reactive oxygen species (ROS) are known to be involved in human reproduction acting as physiological mediators essential in acrosomal reaction or embryo development. However, when ROS outnumber antioxidant defences, they could impair reproductive processes. In particular, at follicular level, ageing-associated oxidative stress has been shown to interfere with this complex microenvironment impairing maturation. In order to counterbalance oxidative insults follicules present several lines of defences constituted by low molecular weight antioxidants, associated with lipoproteins, and enzymatic antioxidants produced by granulosa cells. The ageing process has been shown to affect antioxidant content and total antioxidant capacity. In particular the concentration of enzymatic antioxidants has been shown to increase with age; this has been interpreted as a compensatory mechanism associated with enhanced oxidative stress or as a result of non specific release of cellular content from senescent dying cells. In particular, lypophilic antioxidants deserve particular attention in light of the peculiar lipoprotein sieving occurring at the follicular-blood barrier level during folliculogenesis.

Beta-cryptoxanthin levels have been shown to be associated with embryo grading in IVF-ET procedures and we recently demonstrated that CoQ10 levels resulted significantly higher in mature versus dysmorphic oocytes. Similarly levels resulted significantly enhanced in grading I-II versus grading III-IV embryos. In the present study we show that CoQ10 administration resulted in an elevation of CoQ10 follicular fluid content with Potential implication in oocyte maturation. that might support the supplementation of subjects in late reproductive status.

Ubiquinol supplementation in the elderly patients undergoing aortic valve replacement: biochemical and clinical effects

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There is a steady rise in the mean age of patients affected by heart disease undergoing cardiac surgery. On the other hand senescent myocardium has reduced tolerance to ischemic stress and there are clear indications about age-associated deficit in myocardial performance after operatory stress. Coenzyme Q10 has been shown to ameliorate several conditions related to bioenergetic deficit or increased exposure to oxidative stress. The use of ubiquinol-10, the reduced form of CoQ10, is particularly promising in these clinical settings, on the basis of its superior bioavailability and of the alleged impaired CoQ10-reducing capacity in the elderly.

Here we show the preliminary results relative to 26 patients where we investigated clinical and biochemical effects of ubiquinol-10, administered in the perioperatory stage to elderly patients undergoing aortic valve replacement. Patients, affected by severe aortic stenosis, were randomized into 2 groups: one received placebo, the active group was given 400 mg/die of ubiquinol-10 (QH absorb, Jarrow Formulas, LA, USA) divided into 2 doses, starting 7 days before surgery and continuing for one month after cardiac intervention. Blood samples were withdrawn at time 0 before ubiquinol administration, one day before surgery, 1, 5 and 30 days after surgery. Primary endpoints were the following:

1. Plasma levels of CoQ10

2. Redox status of plasma CoQ10 throughout the study phases

3. Plasma concentration of IL-6, TNF-alpha, S100 protein

Secondary endpoints were:

1. Major cardiac adverse effects in the postoperatory phase

2. Quality of life

3. Evaluation of contractility and myocardial hypertrophy

Results obtained in a first group of treated patients and placebo controls indicate an increase in the percentage of oxidized CoQ10 in plasma following surgical intervention. Treatment with ubiquinol-10 was able to improve basal (before surgery) oxidative status of CoQ10 and to considerably mitigate increased oxidation related to the operation.

Lipid nanoparticles (NLC) loaded with CoQ10: effect on human dermal fibroblasts under normal and UVA-mediated oxidative conditions

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Nanostructured lipid carriers (NLC) represent an emerging tool for drug delivery and are characterized by important features which promote increased bioavailability and epithelial penetration of lipophilic compounds. However, despite these advantages, their potential cytotoxicity should not be underestimated, especially under *in-vivo* usage conditions. Here we analyzed the viability, intracellular reactive oxygen species (ROS), oxidative DNA damage and mitochondrial functionality in human dermal fibroblasts (HDF) in the presence of NLC either empty or loaded with the reduced or oxidized form of Coenzyme Q_{10}. Experiments were carried out under standard culture conditions and under oxidative stress induced by UVA irradiation. The data show that NLC alone produce a slight, though significant decrease in cell viability associated with enhanced oxidative stress. These effects were amplified after UVA irradiation. However, increased ROS levels did not lead to oxidative DNA damage nor mitochondrial impairment. Reduced CoQ_{10}-NLC, differently from oxidized CoQ_{10}-NLC, were able to efficiently counteract UVA-associated mitochondrial depolarization suggesting a potential role of this molecule in antiageing cosmetological formulations. In conclusion, our results suggest that interactions of NLC with cells and biomolecules should be routinely assessed for understanding their compatibility and toxicity, not only under normal conditions, but also under any chemical or physical stress which these delivery systems might be subjected to during their employment.

Nutraceutical bioactive compounds in the prevention of chronic/degenerative diseases

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It is widely accepted that oxidative injury and inflammation are intimately involved in the aging process and the development of age-related diseases. Among the age-related diseases, cardiovascular diseases (CHD) represents the leading cause of death in industrialized and developing countries. Although we lack the power to change some risk factors such as family history, sex or age, there are some key prevention steps we can take. CHD should be targeted as a major set of preventable causes of illness, with effects not only on premature mortality but also on well-being in older ages. Many intervention and epidemiological studies demonstrated that following a heart-healthy diet may prevent or delay the onset of CHD. There is ***convincing evidence*** that increasing consumption of vegetables and fruit reduces the risk of disease. These are the main constituents of the Mediterranean diet (UNESCO Intangible Cultural Heritage of Humanity), characterized by high levels of ***nutraceutical*** bioactive compounds. Nutraceutical, a term combining the words "nutrition" and "pharmaceutical", is a food constituent, mainly a phytochemical component, that provides health benefits, including the prevention and treatment of disease. So, a healthy dietetic approach specifically formulated for elderly people, with a defined pattern of nutraceutical bioactive compounds, may represent a key strategy to improve the aging process.

To date, most dietary anti-aging and cardioprotective strategies have only focused on the delivery of exogenous antioxidants to boost antioxidant status in an effort to protect against toxicant-induced oxidative and inflammatory stress as a means to prevent or combat the negative effects of age-related diseases. A promising new strategy intends to identify nutraceutical bioactive compounds with the ability to directly target and enhance intrinsic cytoprotective mechanisms, including modulation of the expression of genes involved in the detoxification of xenobiotics and their metabolites, genes involved in the synthesis and regulation of intrinsic antioxidants and antioxidant enzymes, genes involved in the regulation of inflammation. In particular, nutraceutical compounds as quercetin, the main polyphenol of the western diet, abundant in apples and onions, may prevent heart damage by inducing multiple cytoprotective pathways, upregulating antioxidant and phase II enzymes, intercepting and detoxifying damaging compounds, and efficiently removing said toxicants before they can initiate further damage (1-3). One more interesting compound is sulforaphane, derived from the hydrolysis of the Cruciferous vegetables glucosinolates, that is able to modulate the expression of genes and proteins related to the Nrf2/phase II detoxification and inflammatory pathways in cardiomyocytes, acting as a "second level" antioxidant (4,5).

But individual nutraceuticals can have greater or lesser effects on specific Nrf2- and inflammation-related genes in various tissues and experimental models. Therefore, only using a combination of nutraceuticals, as those naturally present in fruits and vegetables, it would be possible to modulate the greatest diversity of Nrf2- and inflammation related genes in the greatest number of tissues to achieve the most dramatic protective effects against oxidative damage, toxicants, and inflammation, and to provide the most robust preventive/protective and anti-aging benefits.

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Exercise training ameliorates oxidative stress and inflammatory status in aging and neurodegeneration process in skeletal muscle

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The reduction or lack of physical activity in aging of human skeletal muscle may lead to decreased activities of mitochondrial enzymes, increased generation of reactive oxygen species (ROS)-mediated toxicity leading to proteins, lipids and DNA oxidation as well as mtDNA deletions and mutation. Simultaneously, physical inactivity is associated with elevated pro-inflammatory cytokines in serum that can lead to poor physical performance and muscle strength.

Based on the results of published papers, a hypothesis has been put forth that an exercise training protocol would counteract the changes that have been observed in muscle derived from diseased humans and animals, as well as in aging. However, it seems that in a pathologically altered muscle where there is overproduction of ROS and the associated consequences, exercise training would further increase free radical damage to cell structures and intensify the degeneration of muscle.

However, our studies have shown that resistance training, as well as endurance of low-and medium-intensity exercise leads to a reduction in oxidative stress in aging and dystrophic skeletal muscle. Furthermore, we have observed that an exercise training protocol improves the antioxidant defense system, which causes the muscle to become more resistant to oxidative stress conditions induced by high dose gamma-radiation.

In summary, these results demonstrate that resistance training and endurance of low and medium intensity exercise has a protective effect on aging, dystrophic and skeletal muscle exposed to oxidative stress.

How the antioxidant activity of tea is affected by steeping time, temperature and manufacture

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Tea is second only to water as the most highly consumed beverage worldwide and its medicinal and health properties have been widely explored. It is prepared from the young, tender leaves of *Camellia sinensis (L.)* (family *Theaceae*) which undergo different manufacture procedures to give various types of teas: black, green, white, and oolong. In addition, the growing season, geographical region, processing of the leaves and whether teas are blended or unblended, create the vast selection available commercially and contribute to each tea's uniqueness. Considering the increasing interest in the health properties of tea, unrelated in part to its antioxidant activity, and the different methods of making a cup of tea in different countries and cultures, the present study aimed to discover, whether the antioxidant activity of different tea infusions could be affected by steeping time, temperature and manufacture.

At first, different tea infusions (white, black, green, oolong) coming from different regions of the world were prepared by hot infusion at 90°C for 7 min or by a cold one at room temperature for 2 h, and analyzed. Analysis of the antioxidant activity of these infusions showed that the white tea, unlike black, oolong and green tea, exhibited a greater activity when steeped for 2 h in water at room temperature. We therefore wanted to verify if this feature could be common to most white teas, whose beneficial effects are often attributed to their antioxidant activity and which has been barely studied to date.

Accordingly, a batch of eight white teas was analysed: five from China and three from Malawi (Africa). The teas were prepared according to the typical hot brewing method for white tea (70°C for 5-7 min) and at room temperature for 2 h, and antioxidant activity, chelating activity and overall content of polyphenols, flavanols, and catechins (HPLC) were measured. In addition, to verify if the extraction of antioxidant compounds was affected by steeping time and temperature, some of the studied white teas and their corresponding green teas were extracted at different times and temperature and antioxidant activity, polyphenols content and flavanols of the infusions were determined.

We also aimed to determine whether the manufacturing process of tea affects its antioxidant activity, metal chelating activity and total phenol content (TPC) when variables which influence tea components (geographical region, environmental conditions, cultivar type, plucking techniques) are kept to a minimum. For this purpose, a set of five Malawian teas were analyzed, all originating from the same tea estate and same superior cultivar but whose leaves (grown and plucked under the same conditions) were processed differently in the same factory to give black (orthodox and CTC), white and green (decaffeinated and non-decaffeinated) teas.

The results of these studies contribute to gaining further knowledge on how the potential health benefits of this popular beverage may be maximised by the different methods of preparation and manufacture.

High fat diet -induced met-hemoglobin formation in rats prone (WOKW) or resistant (DA) to the metabolic syndrome

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As there is strong evidence for inflammation and oxidative stress involvement in diabetes and obesity, the aim of this study was to elucidate what is the relationship between oxidative imbalance and red blood cells response as methemoglobin formation in obese/diabete and lean rats.

The Wistar Ottawa Karlsburg W (WOKW) rats develop the main features of the metabolic syndrome characterized by obesity, hypertensions, dyslipidemia, insulin resistance and impaired glucose tolerance. On the other hand, the dark agouti (DA) strain does not show any of these characteristics and has been considered as the control strain for the WOKW rats. Therefore the present study determined the effect of high dietary fat (HFD) on the redox state of haemoglobin in erythrocytes of these two different strains of rats. Moreover, it was tested the effect of Coenzyme Q10 (CoQ10) supplementation in light of its beneficial properties in cardiovascular disease associated to its antioxidant activity. Such supplementation produced a remarkable increase in plasma CoQ10 content, both in WOKW and DA rats, although no significant differences were observed between rats of the same sex in different strain. Notably WOKW rats showed a trend toward a more pronounced increase in CoQ10 plasma levels, almost approaching a significance level in male rats ($p=0.08$).

After a month of HFD intake the methemoglobin formation in WOKW male rats increased significantly and a supplementation of CoQ10 30 mg/kg decreased significantly this value. In DA male rats the HFD intake increased methemoglobin formation but the effect was not significant. In any case this small increase was also reduced by CoQ10 supplementation.

The present data suggests that a diet rich in saturated fat increases methemoglobin formation and CoQ10 supplementation could reduce this formation. The index of methemoglobin formation could be therefore taken as a value of the erythrocyte antioxidant capacity and this could represent a strategy to evaluate the antioxidative status of other tissues.

Effectiveness of a CoQ_{10} solution for ophthalmic use

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Studies on the ocular effects of CoQ_{10} stem from the original observations of Capaccioli et al. (2003) who showed, working with a rabbit corneal keratocyte cell line exposed to different apoptotic stimuli, that CoQ_{10} significantly reduced apoptotic cell death, attenuated ATP decrease, and hindered DNA fragmentation elicited by all apoptotic stimuli. This was accompanied by inhibition of mitochondrial depolarization, cytochrome c release, and caspase 9 activation. Since these events are consequent to mitochondrial permeability transition pore opening, it was suggested that the anti-apoptotic activity of CoQ_{10} could be related to its ability to prevent this phenomenon. Following these initial observations several studies were conducted focused on the effects of topical CoQ_{10} formulations (Coqun® and Visudrop™, Visufarma, Italy) in accelerating corneal epithelial wound healing after several experimental injuries. An in vitro study on epithelial corneal cells (HCE line) irradiated with UVB showed that CoQ_{10} was able to significantly counteract cell mortality and to improve cell vitality. Moreover, in HCE cells exposed to UVB Visudrop™ was capable of maintaining mitochondrial functionality, in terms of respiratory activity and ATP levels. A parallel in vivo study on rabbit cornea after alcohol-mediated disepithelization and corneal scraping highlighted that treatment of rabbits with Visudrop™ enhanced corneal re-epithelization in vivo as measured by the fluorescein technique. The most recent clinical study along this research line (Fogagnolo et al. Ophthalmologica 2013) evaluated the post-operative effects of CoQ_{10} + vitamin E (Coqun®) on the recovery of integrity of the ocular surface after cataract surgery. This formulation was able to promote faster nerve regeneration, compared to controls treated with saline, measured as both temporal and central nerve density and faster recovery of signs and symptoms of ocular surface disease.

Another line of research addresses bioenergetic-based neuroprotection in glaucoma.

Although the progression of glaucoma can be impeded by intraocular pressure-lowering strategies, biochemical interventions aimed at protecting the optic nerve and/or the retinal ganglion cells are also developed, in order to prevent the evolution to blindness. Different molecules have been proposed regarding the achievement of neuroprotection, including CoQ_{10}. A study conducted by Nucci et al. (2007) showed that topical treatment with CoQ_{10} prevents retinal ganglion cell loss in a rat model of high intraocular pressure-induced retinal ischemia. This effect was accompanied by CoQ_{10} capacity of minimizing glutamate increase induced by ischemia/reperfusion. Recently a study by Parisi et al. (J. Glaucoma 2012) evaluated pattern-evoked retinal and cortical responses (PERG) and visual-evoked potential (VEP) after treatment with CoQ_{10} in conjunction with vitamin E in open-angle glaucoma (OAG) patients. Results suggest that this treatment may induce an enhancement in PERG responses in OAG patients. Therefore a possible effect of CoQ_{10} on the innermost (ganglion cells and their fibers) retinal layers function of AOG patients can be assumed. A possible interaction between CoQ_{10} and vitamin E cannot be excluded. In the presence of an unchanged retinocortical time, an index of post-retinal neural conduction, also revealed in this study, improved visual cortex response must necessarily be ascribed to better retinal function.