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Plenary and Award Lecture Abstracts

SFRR-Europe Annual Award Lecture

Redox Biology and Metabolism in Brain Aging and Alzheimer's Disease

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The gradual decline in energy metabolism during brain aging and some neurodegenerative disorders results in a hypometabolic state, which is a function of deficits in (*a*) substrate supply, (*b*) mitochondrial catalysis and energy transduction, and *b*) cytosolic metabolic, signaling, and transcriptional pathways. Mitochondria play a central role for they integrate several signaling processes and generate molecules such as H_2O_2 that coordinate cytosolic signaling and transcriptional pathways through thiol/disulfide exchange mechanisms.

The hypometabolic state inherent in brain aging and a mouse model of Alzheimer's disease was examined in a model that integrates mitochondrial function, insulin signaling, and JNK signaling as well as the effects of nutraceutical interventions –likely acting through thiol/disulfide exchange mechanisms– in the regulation of these parameters. Brain aging proceeds with a decrease of glucose uptake (dynamic microPET imaging), which was associated with a decrease in the expression of the insulin-sensitive neuronal GLUT3/4 and microvascular endothelium GLUT1 (55 kD) but not astrocytic GLUT1 (45 kD). Brain aging was associated with an imbalance between the PI3K/Akt pathway of insulin signaling and JNK signaling and a down-regulation of the PGC1α-mediated transcriptional pathway of mitochondrial biogenesis, thus impairing on several aspects of energy homeostasis. Of note, these effects were observed in cortex- and hippocampal preparations but they are not necessarily cell specific, for astrocytes respond in a different manner: astrocytes showed an age-dependent increase in mitochondrial oxidative metabolism (respiring either on glucose or glucose plus pyruvate) and mitochondrial biogenesis. These metabolic changes were associated with an age-dependent increase in H_2O_2 generation (largely ascribed to NOX2) and NFκB signaling as well as augmented responses with age to inflammatory cytokines. Further, these inflammatory cytokines, IL-1β and TNFα stimulated mitochondrial oxidative metabolism and mitochondrial biogenesis in astrocytes.

Impaired glucose uptake (accumulating into energy deficits) and synaptic plasticity are the major characteristics of the triple transgenic mouse model of Alzheimer's disease. These effects were accompanied with diminished membrane translocation of GLUT4 and GLUT3, increase in tyrosine phosphorylation of the IRS (inactivation) and decrease in serine phosphorylation (activation). The former effect coincided with increased activation of JNK1 and diminished activation of Akt. The JNK/Akt imbalance affected mitochondrial energy metabolism. The functional outcome of this hypometabolic state was translated into a substantially diminished long-term potentiation.

The hypometabolic state in both models, brain aging and the mouse model of Alzheimer's disease, was rescued by treatment with lipoic acid, likely through a thiol/disulfide mechanism, that resulted largely in activation of the insulin receptor substrate and higher translocation to the membrane of the insulin-sensitive glucose transporters. In this manner, lipoic acid treatment appears to increase substrate supply; its effectiveness resulted in an increase of synaptic plasticity measured as long-term potentiation.

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Catherine Pasquier Memorial Award Lecture (shared)

Critical role of oxidative and nitrosative stress in pathophysiology: Inflammation, cell injury and cancer

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The presence of chronic inflammation associated with sustained cell death and proliferation turnover is a potential tissue environment for cancer development. In this situation, oxidative and nitrosative stress are the major driving force playing a key role either as a preventing or inducer/promoter of tumorigenesis. During inflammatory response, activated macrophages release a great variety of protease that degraded extracellular matrix, inflammatory mediators that plays a relevant role in chemotaxis and cell signaling, as well as oxygen and nitrogen reactive species (ROS and RNS, respectively) actively involved in cell defense through oxidation of essential biological molecules such as DNA, carbohydrates, proteins and lipids. Iron-loosely bound plays a relevant role in promoting oxidative-dependent reactions leading to cell damage. In this sense, we have shown that locally iron overload induced an accumulation of neutrophils with increased NADPH-derived superoxide anion generation in exudates from rats submitted to carrageenan-induced granuloma model. The increase of lipid peroxidation products was associated with

the presence of iron-loosely bound, reduction of antioxidant status in inflammatory exudates and blood, as well as induction of hepatic acute phase response. A similar pro-inflammatory and oxidant reaction was observed by intraperitoneal iron-dextran administration in animals with *Mycobacterium butyricum*-induced adjuvant arthritis. The administration of a diet devoid of polyunsaturated fatty acids reduced the substrate for lipid peroxidation, and consequently a decrease of conjugated diennes and thiobarbituric acid-reactive substances in plasma and hepatic microsomal fraction were observed in animals with adjuvant arthritis. The acute phase response induced by different inflammatory mediators in hepatocytes has been studied in detail. The induction of carrageenan-induced granuloma drastically reduced the expression of drug metabolizing enzymes (DME) (CYP3A1, CYP4A, CYP2B1, CYP2D and CYP2E1) and their related-activities in rat livers. Cytokines (IL-1α, IL-6 and TNF-α) exerted a specific timely-dependent regulation of DME expression (CYP1A1, CYP1A2 and CYP3A4) and secretion of acute phase reactants (albumin, ferritin, fibrinogen and ceruloplasmin) in cultured primary human hepatocytes. Although TNF-α induces acute phase response and liver injury in different experimental settings such as sepsis, we have also shown that TNF-α-dependent induction of nitric oxide synthase type II (NOS-2) hepatic expression during PGE_1 pre-administration drastically reduces D-galactosamine (D-GalN)-induced liver injury in rats. The pre-administration of PGE_1 also reduced cell death induced by D-GalN in primary culture of human and rat hepatocytes. However, the cytoprotective properties of PGE_1 do not involve a reduction of D-GalN-induced ROS production. Nitric oxide (NO) has demonstrated to exert pro- and anti-apoptotic properties according to local concentration, target cell, as well as the presence of other ROS such as superoxide anion generating high reactive peroxynitrite. We have observed that moderate increase of PGE_1-dependent NO prevented further potent NF-kB-dependent NOS-2 expression and apoptosis induced by D-GalN in cultured rat hepatocytes. However, the administration of PGE_1 at advanced stages of cell injury or high doses of NO donor increased cell death in D-GalN-treated human and rat hepatocytes. The induction of apoptosis by D-GalN was associated with ROS production, mitochondrial dysfunction and post-translation (S-nitrosylation and Tyrosine nitration) modifications of proteins involved in unfolded protein response, enzymatic antioxidants, cell metabolism and death in cultured human hepatocytes. In consequences, the administration of antioxidants such as N-acetylcysteine, Q10 and MnTBAB reduced ROS generation, mitochondrial dysfunction and apoptosis induced by D-GalN and glycochenodeoxycholate (GCDCA) in cultured human hepatocytes. The cytoprotective properties of α-tocopherol appears to mostly related to gene regulation (NOS-2, PPAR-α and carnitine palmitoyl transferase) rather than to its antioxidant activity in D-GalN-induced cell death in hepatocytes. The reduction of cell death by α-tocopherol was also associated with reduction of CYP7A1, Na^+-taurocholate co-transporting polypeptide (NTCP), and heme oxygenase-1 expression, as well as increased NOS-2 expression and NO production in GCDCA-treated hepatocytes. α-Tocopherol and NO donors increased NTCP cysteine S-nitrosylation and Tyrosine nitration, and reduced Taurocholic acid uptake in hepatocytes. The pro-apoptotic properties of high NO donor concentration have been successfully applied to exert antitumoral activity in liver cancer cells. In this sense, the administration of NO donor or NOS-3 overexpression induces p53-dependent cell death receptor expression and apoptosis in hepatoma cell lines. The first generation adenovirus specifically designed to induce NOS-3 overexpression in liver cancer cells induced oxidative and nitrosative stress, DNA damage, p53, cell death receptors expression and their S-nitrosylation, and apoptosis that was correlated to a reduction of cell proliferation and growth of tumors orthotopically implanted in fibrotic livers. The S-nitrosylation of cell death receptors (TNF-α, CD95 and TRAIL-R1) plays a critical role on the shift from extrinsic to intrinsic cell death pathways during antitumoral molecular therapy (Sorafenib Tosylate) in liver cancer cells. In conclusion, new data are emerging that elucidate the extraordinary role that plays ROS- and RNS-dependent posttranslational target modifications in regulating intracellular cell signaling.

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Catherine Pasquier Memorial Award Lecture (shared)

The adventure of detecting subtle signals (such as protein nitrosothiols and superoxide) among cell traffic jams (from proteomics to hypoxia)

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Like big human cities, cells are crowded environments with a lot of inhabitants, where a myriad of signals try to orchestrate how each component can do its best among the crowd, and how the whole system respond to changes in the environment. Some signals are robust and stable, so they can be easily studied by life science researchers. However, free radical researchers know well that other molecular signals are short-lived and unstable. I have been involved in several studies that can be seen as adventures searching for those subtle signals, overcoming the difficulties that appear in the way.

One is the reliable proteomic identification of protein S-nitrosylation (or S-nitrosation), and of other reversible oxidations of cysteines, in physiological scenarios where they can act as signals (not just in nitrosative, oxidative or similar stresses). Different techniques have been used for that purpose (called “thiol redox proteomics”), with technical developments still ongoing. A related adventure is the study of the functional relevance of these modifications in individual proteins, and of the particularities of these modes of signalling.

Another adventure deals with the role of reactive oxygen species production in hypoxia sensing and adaptation, on which a debate has been maintained for a long time. We have measured superoxide production in acute hypoxia, showing there is a fast burst at the first minutes of acute hypoxia, and we are currently studying its molecular mechanism, implying mitochondrial complex I and the sodium/calcium exchanger. This can be a common signal to different cell types that produces diverse effects depending on the signalling cascades and effectors of each cell type. Thiol redox proteomics can help in identifying the specific protein cysteine residues that can be affected by reversible oxidation and take part in those signals.

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SFRR Europe Clinical Science Lecture - Sponsored by DSM

Protein misfolding in the ER and oxidative stress synergize to promote cell death

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SFRR Europe Basic Science Lecture - Sponsored by Elsevier

Untangling H_2O_2 toxicity from its regulatory functions using 2-Cys peroxiredoxins and linked reductases network

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SNFS Advances in Nutrition Lecture - Sponsored by SNFS

Antioxidants and human disease, the value of cell culture and animal models

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Oxygen radicals and related "reactive oxygen species" (ROS) are fundamental to human life; they help drive evolution and the damage that they can do ("oxidative damage") is involved in most, if not all, human diseases, especially neurodegenerative diseases and stroke, and in ageing itself. Nevertheless, ROS have beneficial effects in killing invading organisms and facilitating signal transduction, especially in coordinating the inflammatory response.

In establishing the role of ROS in disease, frequent use is made of animal models and of cell culture. Yet animal models have rarely been predictive of the outcome of clinical trials of antioxidant drugs in human disease, especially the neurodegenerative diseases and stroke. Reasons for this will be explored, but our studies on human stroke patients may illustrate one reason why.

Even more questionable is cell culture. Cell culture is an example of hyperoxia-induced oxidative stress and how cells adapt to it, mutating rapidly and changing their metabolism. Culture media often interact in complex ways with added "antioxidants", sometimes catalyzing their oxidation to H_2O_2 and other cytotoxic species. Hence one must take great care to avoid artefacts when interpreting the effects of added antioxidants or other compounds upon cells in culture. The apparent ability of ascorbate and polyphenols to kill cancer cells is one such artefact.

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