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Meeting Report

Redox regulation and the metabolic syndrome

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XIth Villa Vigoni Conference 'Redox regulation and the metabolic syndrome'. Villa Vigoni, Loveno di Menaggio, Italy, 23–26 March 2011

After almost 25 years, it is now the XIth Villa Vigoni conference held at Lago di Como in Northern Italy to focus on redox regulation, this time linked to the metabolic syndrome. Villa Vigoni was again an excellent location for scientific discussions given by 24 speakers (Figure 1). Besides Italian and German scientists, for the first time the XIth Villa Vigoni conference turned into a forum for 'redox enthusiasts' at a European dimension.

Redox Signaling

First, how the concept of redox reactions as part of a 'stress response' evolved into that of genuine 'physiological controllers' of cell functions was presented. An equilibrium between signaling and toxicity of reactive oxygen species (ROS) can be basically explained by chemical reactions following a 'two electron' or a 'one electron' oxidation pathway, respectively. Thiol redox homeostasis was then discussed, exploiting genetic tools to demonstrate that in yeast glutathione (GSH) is essential for life, though concentrations above normal provoke reductive stress, elicit an unfolded protein response and cell death. Although GSH regulates iron metabolism, it is thioredoxin that maintains the cytoplasmic thiol redox balance. The effect of tyrosine nitration in insulin resistance was then presented to show that the protein kinase B/Akt-signaling impacts on glucose transporter 4 (GLUT4) translocation in myotubes. Angiotensin II blocked GLUT4 translocation by producing peroxynitrite ($ONOO^-$), thus nitrating and inhibiting Akt, both *in vitro* and *in vivo*. Redox regulation, and particularly the role of nitric oxide (NO), was discussed for endothelial cells with reference to post-translational protein modifications by tyrosine nitration, *S*-nitrosylation and *S*-glutathionylation. Redox perturbations initiated by cyclosporine A caused manganese superoxide dismutase nitration, thus causing its inhibition and subsequent mitochondria-dependent death. Plasma membrane redox systems were then reviewed to describe the role of NADPH oxidases (Nox1-5 and Duox1-2), NAD(P)H:quinine oxidoreductase 1 (NQO1), disulfide–thiol exchangers, voltage-dependent anion selective channels 1–3, duodenal cytochrome *b*, and cytochrome b_5 reductase. Alterations of these carriers in obesity, cardiovascular diseases and insulin resistance were discussed. The redox signaling session ended with a talk on MAPK-dependent signaling. By using knockout mice for p38 MAPK, it was shown that the δ form is specifically involved in inhibiting insulin secretion by blocking exocytosis in β cells. This effect occurred through an inhibitory phosphorylation of protein kinase D1, thereby modulating *trans*-Golgi network.

Mitochondrial Function

The second session moved to mitochondria, organelles recognized for redox reactions. First, the role of modulating Nox activity by apocynin in experimental models of spinal cord injury (SCI) and cerebral ischemia/reperfusion was addressed. Apocynin reduced free radical formation, prevented the reduction of IkB-α, blocked accumulation of adhesion molecules, and lowered SCI-induced apoptosis. Second, modulation of mitochondrial function by lipolysis was on stage. Lipolysis requires the activity of adipose triglyceride lipase (ATGL). *Atgl*−/− mice show a massive accumulation of triglycerides, severe cardiac phenotype and a twofold larger white adipose tissue compared with wild-type littermates. Interestingly, ATGL had a distinct role in the release of fatty acids from intracellular lipid droplets, thus allowing them to activate peroxisome proliferator-activated receptor α (PPARα), which enhanced mitochondrial biogenesis and function. In line, cardiac mRNA levels of virtually all PPARα targets were reduced in *Atgl*−/− mice and reintroduction of ATGL in the heart of *Atgl*−/− animals was enough to afford a normal respiration and to prolong the life span. Finally, kinetic aspects of modulating mitochondrial respiration by NO were addressed, presenting two inhibition mechanisms that lead to accumulation of either nitrosylated cytochrome *c* oxidase or

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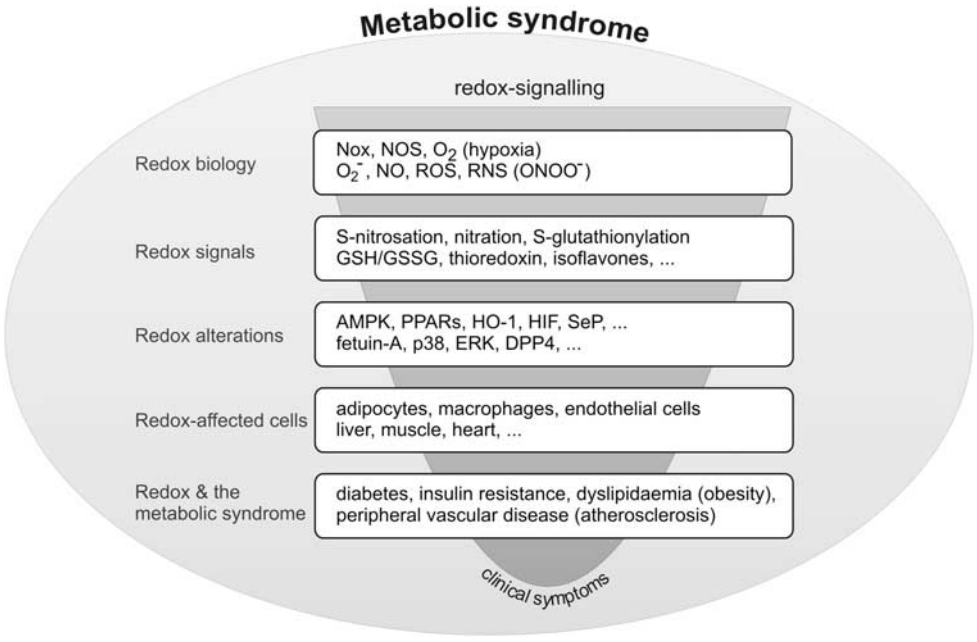

Figure 1 Redox signals in the metabolic syndrome. The figure summarizes major aspects discussed during the meeting that cover distinct control levels, thus bridging from redox biology to clinical symptoms. See the text for details and abbreviations

nitrite. The first mechanism is O_2 sensitive and has a high turnover but a slow recovery, whereas the second is O_2 insensitive and reveals a low turnover but fast recovery. The choice between these mechanisms depends also on melatonin, which regulates nitrosative stress due to NOS activity.

Leukocyte Regulation

Initially, differentiation of macrophages to foam cells was described to demonstrate that interferon-β is downregulated during this process, thus blocking interleukin-10 (IL-10) formation. Interestingly, IL-10 inhibited atherosclerosis, and mice lacking IL-10 receptor showed reduced circulating cholesterol. Afterwards, the role of macrophage NF-E2-related factor-2 (Nrf2) in atherogenesis was addressed, showing that this transcription factor induces the expression of antioxidant genes, such as heme-oxygenase 1 (*HO-1*) and *NQO1*, with the further suggestion of an antiatherogenic role for Nrf2 *in vivo*. Macrophage foam cell formation with oxidized low-density lipoproteins (oxLDL) was further exploited to investigate Toll-like receptor 4 signaling and ROS formation. In this paradigm, oxLDL provoked Nrf2-mediated activation of HO-1, and inhibition of HO-1 accounts for reduced Ccaat-enhancer-binding proteins β/δ activity. A less known modification of LDL, so-called 'phospholipolyzation', is catalyzed by secretory phospholipases (sPL) such as $sPLA_2$. The PLA–LDL products can activate phsphatidylinositol-3-kinase/Akt signaling in monocytes, thus promoting their survival. The contribution of Bad phosphorylation to the ability of monocytes to survive to $ONOO^-$ was also presented, and was shown to demand arachidonic acid release via cytosolic phospholipase A_2, followed by its oxidation by 5-lipoxygenase and subsequent activation of protein kinase Cα. Phosphorylated and thus inactive Bad will be released in the cytosol in association with Bax and will sustain cell survival. The last lecture addressed the functional status of NO/NOS in human mature and immature neutrophils. Following thrombosis, hypoxia–reoxygenation and hypertension, an increase in NO affects neutrophil migration and their bacterial killing potential. Although inducible NOS expression increases during neutrophil maturation, eNOS was downregulated and nNOS remained unchanged.

Metabolic Syndrome/Obesity

The first lecture was devoted to high selenium intake as an enhancer of diabetes risk. Epidemiological studies suggested a correlation between selenium and diabetes, and indeed selenoprotein P expression interferes with glucose metabolism and insulin signaling, ultimately inducing insulin resistance. Thereafter, mitochondrial ROS and the enzymatic activity of Nox and xanthine oxidase were shown to be markedly enhanced in animal models of type 1 diabetes in which insulin therapy was able to reverse these alterations along a pathway that engaged eNOS. Then fetuin-A (FetA), a secreted cysteine protease inhibitor, was shown to inhibit insulin signaling. FetA serum levels predict cardiovascular risk, and FetA overexpression causes atherosclerosis, weight gain, and insulin resistance in mice. Also adipocyte dysfunction on hypoxia was discussed in the context of obesity. Hypoxia occurs in white adipose tissue and several transcription factors, including hypoxia-inducible factor-1α, are activated. Adipocytes are very sensitive to hypoxia, and their dysfunction can have a role in obesity both at the inflammatory and metabolic interfaces. The focus then shifted to vascular cells in which the regulation and function of α_1AMPK was discussed. α_1AMPK regulates energy consumption and is a putative drug target in the treatment of diabetes. It prevents cell death and thus preserves endothelial function *in vivo*.

Conversely, genetic deletion of α_1AMPK enhances $ONOO^-$ formation, ROS production and Nox2 expression compared with wild-type mice. Next, dietary isoflavones (from soy beans) were on the stage, and their 'estrogenicity' (i.e., their structural similarity with estrogen) was discussed. Isoflavones regulate eNOS transcription and antioxidant defense genes, through ERK1/2- and Akt-dependent phosphorylations. Other factors in cardiovascular protection are prostanoids. Shear stress upregulates cyclooxygenase-2 activity, and hence HO-1 expression in human endothelial cells, which in turn reduces the pro-inflammatory mediator synthesis. Then, a proteomic approach was reported to profile adipocyte-conditioned media, and out of 351 proteins that could be identified in the 'adipocyte secretome', 44 were discovered as new adipokines. One of these, dipeptidyl exopeptidase 4, correlates with the metabolic syndrome parameters, and its inhibitors promise as effective drugs to treat type 2 diabetes. The last lecture covered the role of redox regulation on endocannabinoid signaling. Endocannabinoids regulate energy homeostasis by affecting mitochondria, as well as type-1 cannabinoid receptors at the central (hypothalamus) and peripheral levels (adipose tissue, liver, pancreas and muscle) to stimulate food intake and fat accumulation.

Concluding Remarks

Participants of the XIth Villa Vigoni conference concurred that such a forum for exchanging ideas and fostering new collaborations in the evergreen field of redox regulation is needed. The merge of basic scientists and clinicians was unanimously recognized as an important advantage for a better understanding and potential therapeutic exploitation of redox reactions in several human diseases.

Conflict of Interest

The authors declare no conflict of interest.

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