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Meeting Report

Redox regulation in disease and ageing

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Villa Vigoni Conference 'Redox regulation in disease and ageing': Villa Vigoni, Loveno di Menaggio, Italy, 24–27 March 2004.

The Italian-German Villa Vigoni Conferences on this topic were established over 15 years ago with the aim of offering a forum for discussions on redox-mediated reactions, which affect disease and ageing. The impact of redox reactions on human health has also been the focus of the Eighth Conference, held at the breath-taking Villa Vigoni, Loveno di Menaggio, Italy, as a joint Italian-German event with a total of 27 invited speakers expert in the field.

Molecular oxygen (O_2) is the most common terminal electron acceptor for oxidative phosphorylation, and hence it plays an essential role in many metabolic processes associated with aerobic life. However, a partial reaction of oxygen with electrons leads to the formation of a variety of reactive oxygen species (ROS), which may cause extensive damage to cells. This peculiar condition of aerobic life is known as the 'oxygen paradox' and can be explained by the 'oxidative stress' hypothesis. Many recent reports indicate that oxidation and reduction are also used for regulatory processes. Therefore, reduction of O_2 by cells is a tightly regulated mechanism, and the different sources of oxidative reactions are under the control of a multifaceted antioxidant and reducing system.

Generation and disposal of ROS

A free radical is a species with one or more unpaired electrons, thus O_2 is itself a biradical, with two unpaired electrons each located in an antibonding orbital. Several biologically important reactions can increase the reactivity of O_2, yielding reactive species like O_2^-, H_2O_2, $^1O_2$, or the OH-radical (ROS). It is commonly accepted that under physiological conditions mitochondria are the major site of intracellular generation of ROS, due to electron leakage along the respiratory chain. In addition, autoxidation or free radical chain reactions (e.g., hydrogen abstraction, electron transfer, addition, termination and disproportionation) of biological molecules like carbohydrates, proteins, lipids, RNA and DNA may account for a significant amount of ROS, particularly in the case of lipids. Indeed, reaction of polyunsaturated fatty acid hydroperoxides with metal complexes generates lipid alkoxyl radicals, which are presumed to start metal-amplified lipid peroxidation of biological membranes. On the other hand, aerobes must develop an effective defence against ROS in order to exploit O_2 for respiratory energy production. This defensive system includes: (i) a complex array of antioxidant enzymes able to scavenge and detoxify reactive species, like superoxide dismutases (SOD), catalase, DT diaphorase, glutathione peroxidase, glutathione reductase and thioredoxin reductase; (ii) high- and low-molecular-weight breakers of free radical chain reactions, like thioredoxin, tocopherols, carotenoids, ubiquinone, ascorbate, glutathione and urate; and (iii) high-molecular-weight trappers of transition metals such as lactoferrin, transferrin, ferritin and caeruloplasmin.

The balance between ROS generation and disposal, rather than the absolute amount of a single oxidant/antioxidant species, appears to determine the physiological and pathological effects of free radicals, and the impact of redox regulation on cell functioning. On this issue, a number of hot topics emerged in the centre of intense discussions at the 2004 Villa Vigoni conference:

The differences between 'oxidative stress' and 'redox regulation' or 'redox signalling' have become clearer with ongoing research. Many events associated with oxidative stress have been triggered with exogenously added oxidants like H_2O_2, organic peroxides, radical sources or simply hyperbaric oxygen. Typical read-outs were parameters of lipid peroxidation, protein carbonyls or loss of certain cell functions. Characteristically such changes are irreversible and eventually are leading to necrotic or apoptotic cell death. In contrast, redox signalling affects regulatory proteins through oxidations of thiols to mixed disulphides or *S*-nitroso derivatives, vicinal dithiols to disulphides, zinc-fingers to disulphides or methionines to methionine sulphoxides. Such modifications can lead to activity changes in a reversible

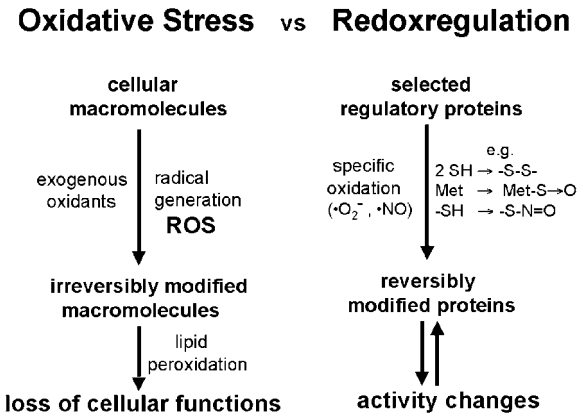

Figure 1 A change of perspective from oxidative stress to redox regulation. Changes associated with oxidative stress are irreversible and eventually lead to necrotic or apoptotic cell death. In contrast, redox regulation (or redox signalling) affects regulatory proteins through various oxidations, which ultimately lead to activity changes in a reversible manner, and hence are the basis for cell regulation

manner, and hence are the basis for cell regulation, as depicted in Figure 1.

Certainly, there are transitions between redox regulation and oxidative stress, like in ischaemia/reperfusion, diabetes, atherosclerosis or neurodegenerative diseases. Only by knowing the mechanisms for such pathological events a meaningful therapeutic approach can be proposed.

New insights into ROS activity

NO and its derivatives are known to act directly on proteins at cysteine residues, but also tyrosines can be important targets for nitration (Ullrich). In addition, NO causes nuclear translocation of transcription factors and can act as an intercellular mediator (Brüne). The latter action and the known instability of NO leave open the intriguing question of how long NO can last in the extracellular environment. Should we consider NO a short-range mediator, produced on demand in the right spot and at the right time, analogously to eicosanoids? Or are there mechanisms which prolong its lifespan, and hence its biological effects?

Also, superoxide has been shown to modulate signal transduction, by inhibiting calcineurin in a reversible, and hence physiologically relevant manner (Namgaladze). In addition, it has been implicated in turning 'redox signalling' into 'phosphorylative signalling' along apoptotic pathways, through thiol/disulphide reactions on mitochondrial membrane proteins (Filomeni). Furthermore, the use of a new generation of drugs called 'SOD mimetics' (SODm) has allowed to demonstrate that superoxide anion is critical for the regulation of septic and nonseptic shock (Cuzzocrea). There are, however, questions pending, which basically concern the cell specificity of the intracellular transduction of redox regulation and the contribution of membrane lipid peroxidation to the effects of ROS on membrane proteins. The balance among these factors might be essential for fine-tuning the cell choice between survival and death. New evidence has been presented on redox control of arachidonate cascade (Cantoni) and of molecular recognition of the arachidonate derivatives 'endocannabinoids' by receptors, transporters and hydrolases (Maccarrone). The data point towards mitochondria as a common 'sensor' of the activity of these lipids on cell signalling. In addition, the action of selenoproteins has expanded in the last few years, demonstrating new roles in controlling embryogenesis and male fertility (Flohé). Also, the mechanisms of ROS generation have gained new insights. Fluorescence imaging has allowed to 'see' ROS formation in lungs under hyperoxia, due to mitochondrial calcium-mediated NADPH oxidase activation (Kübler). In addition, the latter enzyme mediates the membrane potential-dependent formation of oxygen radicals by smooth muscle cells, platelets and endothelial cells in resistance arteries (Pohl), and in these cells also cytochrome P_{450}-dependent arachidonate metabolism has been reported as a valuable source of ROS (Fleming).

ROS in disease and ageing

The meeting has then focused on pathology. Recent advances on the cardiovascular system were presented by Bachschmid, Landmesser and Schildknecht, showing that treatment with extracellular SOD, which is defective in cardiovascular patients, reverses endothelium dysfunction. In addition, angiotensin II triggers the generation of NO, which reacts with superoxide to generate peroxynitrite ($NO + O_2^-$. $ONOO^-$), and as such it activates cyclo-oxygenase-2, ultimately leading to endothelial cell activation. A systemic induction of cyclo-oxygenase-2 has been shown to be a critical factor also for atherothrombosis, again implying regulation by angiotensin II (Cipollone). Still on the vascular system, the mechanism of nitrate tolerance has been demonstrated to be redox regulated by a mechanism whereby mitochondrial ROS formation, nitroglycerin bioactivation and prostacyclin synthase activity are reduced, thus impairing vasodilation of the vessels (Daiber). The muscular system was then addressed, showing that muscle cell differentiation is associated with increased antioxidant ability and that muscular disease is under redox control, especially at the level of thioredoxin reductase expression (Avigliano).

The latest advances on a number of neurodegenerative disorders were also presented. Amyotrophic lateral sclerosis is due to a mutant form of SOD1 (fALS-SOD1), whose expression in motor neurons leads to ROS generation and is linked to proteasome dysfunction (Carrí). The potential involvement of redox regulation in Huntington's disease has been reviewed, basically suggesting that it might take place at two hot spots: control by the neuroprotective protein huntingtin of the expression of BDNF (brain-derived nerve factor), which involves nuclear translocation of specific transcription factors and modulation of calcium release from mitochondria, leading to calpain activation and neuronal apoptosis (Cattaneo). Also, AIDS was discussed, showing that AIDS-associated dementia induced by the HIV-1 glycoprotein gp120 deranges the arachiodonate cascade in favour of the cyclo-oxygenase pathway, at the expense of the lipoxygenase pathway, thus leading to glutamate release, excitotoxicity and neuronal loss (Bagetta). Furthermore, lipid peroxidation and oxidative DNA damage were reported

among the most prominent environmental factors of Parkinson's disease, resulting in excitotoxic insult, mitochondrial dysfunction and activation of NO synthase (Bonsi). Of interest is the fact that in AIDS-associated dementia, Huntington's disease and Parkinson's disease endocannabinoids are neuroprotective and that all these neurodegenerative diseases involve mitochondria as a key point for redox regulation. In fact, alterations in ROS production by these organelles have been clearly shown in a model of cerebellar granule cell apoptosis, where the timing of cytochrome *c* release and its ability to act as an electron donor to O_2 were also demonstrated (Passarella). Further insights into mitochondrial biochemistry were presented showing how NO, which acts as a potent inhibitor of mitochondrial respiration, reduces cytochrome *c* oxidase activity through direct interactions with Fe^{2+}/Fe^{3+} in the active site, leading to nitrosyl/nitrate adducts, respectively (Sarti).

Ageing was on the floor as the last but not the least topic of the conference. The ageing model *Podospora anserina*, a filamentous fungus, was presented in order to show that senescent cells have a copper-dependent mitochondrial DNA instability, whereas juvenile cells do not, overall confirming that mitochondria also play a critical role in ageing (Osiewacz). On the other hand, ageing of mammalian skin has been shown to depend on SOD2 and matrix metalloproteases, leading to profound alterations in mitochondrial architecture (Scharffetter-Kochanek). In mammals maintenance of genomic integrity and longevity has been elegantly shown to depend on poly-ADP ribosylation, PARP-1 acting as a negative regulator of genomic instability in cells under genotoxic stress (Bürkle). How can diet contribute to redox regulation? This issue was addressed in the last lecture, showing that vitamin E, a dietary antioxidant, does regulate gene expression and cellular signalling, possibly through a specific nuclear receptor (Brigelius-Flohé).

Concluding remarks and future perspectives

Converging evidence points towards the importance of free radicals in human disease and ageing, suggesting their potential role and therapeutic utility in the treatment of neurodegenerative diseases, as well as of pathologies of virtually any peripheral system. Mitochondria are essential for both disease and ageing, and they seem to be the converging point of different redox-regulated pathways. In addition, the concept that NO and SOD are the key players of redox regulation, conserved along evolution and across different pathologies, has been strengthened by this conference.

The question arises as to whether or not we can modulate ROS levels through targeted pharmacology. To give an example, free radicals can be produced by UV radiation on melanins, therefore ROS scavengers can be used to enhance the efficacy of sunscreens like creams containing vitamins A and E and pills based on carotenoids. Recent research efforts have been focused on flavonoids, which are free radical scavengers widely represented in foodstuffs. In fact, soybean, propolis, hamamelis and sheabutter are only a few examples of vegetal compounds used in medicine for their flavonoid content. Recently, the antimicrobial action of NO has been exploited in dermatology, and a cream of SNAP (*S*-nitroso-*N*-acetylpenicillamine), a typical NO-donor compound, has been successfully used in the topical treatment of cutaneous mycosis.

Given that free radicals have been shown to play an important role in human disease and ageing, the key to effective treatment of the various diseases lies in the investigation into, and the clinical use of, substances that might prevent the toxicity of ROS, or that might mimic their beneficial properties.

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Further Reading

The reader may appreciate some review articles published in CDD during the past, which cover individual aspects addressed in this meeting report for further information.

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