Free Radical Research, 2003 Vol. 37 (4), pp. 349–354

Meeting Report

2nd International Meeting on Free Radicals in Health and Disease

The role of oxidants and antioxidants in the regulation of chronic diseases, May 8–12, 2002, Istanbul, Turkey

IRFAN RAHMAN[a,*] and ALDO TOMASI[b]

[a]*Respiratory Medicine Unit, ELEGI Laboratory University of Edinburgh Medical School, Teviok Place, Edinburgh EH8 9AG, UK;* [b]*SFRR-Europe Secretary, Dipertimento di Scienze, Biomediche, via Campi 287, 41100 Modena, Italy*

This was the 2nd conference on the role of oxidants and antioxidants in the regulation of chronic diseases; the first meeting was held in Istanbul in 1995. Approximately 200 scientists, clinicians and students attended the meeting. The conference viewed the role of oxidants and antioxidants in the mechanism of the pathogenesis of a wide variety of chronic inflammatory diseases and included a free radical school on the measurement of free radicals, evaluation of oxidant/antioxidant status and clinical applications. There was an interesting round table discussion led by eminent scientists in the field of free radicals. It was noticeable that participation by young researchers was actively encouraged. The meeting was a great success, with four days of outstanding scientific sessions and poster sessions, combined with a social program that provided an opportunity for young scientists to discuss ideas with established investigators during the coffee and lunch breaks. Overall, this was an excellent and well-organized meeting in a beautiful setting and the concept of the organizers was well justified by the outcome.

The opening lecture from **Professor Lester Packer** (Los Angeles, USA) set the scene of the conference by providing an update on the role of oxidants and antioxidants in health and disease. The term "oxidative stress" was defined as "the imbalance that arises when exposure to oxidants changes the normal redox status". It was highlighted that a balance between oxidants and antioxidants must be maintained to minimize the molecular, cellular and tissue oxidative damage. The antioxidant properties of vitamins C and E, thiols such as glutathione, thioredoxin, lipoic acid and polyphenols from food sources or herbal supplements, e.g. Gingko biloba extract EGb 761 etc., were described in health and disease. This became one of the focal points of the ensuing round table discussion.

The opening lecture was followed by a lecture from **Professor Angelo Azzi** (Bern, Switzerland) in which he elegantly highlighted the role and mechanism of α-tocopherol in preventing atherosclerosis. Inhibition of protein kinase C activity by α-tocopherol was shown to be the basis of the vascular smooth muscle cell growth inhibition. Inhibiting the activity of protein kinase C by α-tocopherol results in different events in different cell types including monocytes, macrophages, neutrophils, fibroblasts and mesangial cells. Adhesion molecule expression and inflammatory cell cytokine production were also influenced by α-tocopherol. The important finding of the effect of α-tocopherol against atherosclerosis is not due only to the prevention of LDL oxidation but also to the down regulation of the scavenger receptor CD36 and to the inhibition of protein kinase C activity.

*E-mail: irfan.rahman@ed.ac.uk

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/1071576031000076295

Professor Dimitrios Galaris (Ioannina, Greece) presented new data on the molecular mechanisms of oxidant-induced DNA damage and apoptosis in cultured cells. By using single cell gel electrophoresis (comet assay), rapid formation of single strand breaks in nuclear DNA was shown in cells exposed to hydrogen peroxide. It was concluded that intracellular iron and calcium are important mediators of DNA damage in response to oxidative stress. On the contrary, sustained generation of reactive oxygen species (ROS) inhibited the execution of the apoptotic process. Further studies are in progress to unravel the ROS-mediated apoptosis in primary human lymphocytes. Dr Galaris announced that the next SFRR (Europe) meeting will be held in Greece (details are given below).

Professor Helmut Sies (Düsseldorf, Germany) described elegant studies of selenoproteins as ubiquitous biological antioxidants, and the role of epicatechins on inhibition of leukotriene B4. Selenium is incorporated into proteins as selenocysteine or as selenomethionine. Selenocysteine occurs in the active center of glutathione peroxidases, and is present in thioredoxin reductases and in selenoprotein P. The biological role of selenoproteins is intimately linked to selenium redox chemistry. The selenium containing compound, Ebselen, has been shown to possess both antioxidant and anti-inflammatory properties. Further studies are in progress to determine the role of Ebselen in induction of protective antioxidant enzymes and to further study the role of Ebselen *in vivo*. This important subject of research has comprehensively been published in *Methods in Enzymology* (volumes 347 and 348).

The next speaker was **Dr Tilman Grune** (Berlin, Germany) who described the detailed mechanism of protein oxidation in neurodegenerative diseases. Oxidatively modified proteins are selectively recognized and degraded by the cytosolic 20S proteasome *in vitro* systems. It was shown that moderate levels of oxidative stress are able to significantly increase the degradation of intracellular proteins. In contrast, severe oxidative stress decreased proteolysis rates. The presence of highly oxidized lipofuscin is directly involved in the inactivation of the proteasomal system in human fibroblasts. It was concluded that an accumulation of oxidized/cross-linked proteins might actually cause a further increase in tissue damage during ageing and neurodegeneration by inhibiting the proteasome. It remains to be determined whether phosphatases/kinases or chaperons/ubiquitination play an important role in oxidative inactivation of proteins.

On the second day of the conference, **Professor Dipak Das** (Connecticut, USA) gave an overview of antioxidants and redox signaling in ischemic heart disease. They have found a new paradigm, "a third line of defense", consisting of oxidant-inducible genes and proteins which are generated by the heart in an attempt to counteract the invading ROS in an inducible pathway regulated by a precise signal transduction system. He also presented his ongoing study on a signaling cascade regulated by tyrosine kinase–phospholipase coupled activation of MAP kinases and protein kinase C in transmitting the death signal triggered by myocardial ischemia/reperfusion. The study implicates that antioxidants generated as an adaptive response against stress-induced cardiovascular diseases that play an important role in maintaining the balance between antioxidant reserve and ROS and, thus, dictates the need of antioxidant supplementation to combat the cardiovascular diseases.

The second speaker **Professor Frank Kelly** (London, UK) delivered an excellent talk on oxidative stress in pre-eclampsia and the beneficial role of antioxidant supplementation. He convincingly showed that a reduced incidence of pre-eclampsia in women at risk who received vitamin C (1000 mg/day) and vitamin E (400 IU/day) supplements. They also found that this clinical benefit was associated with improvement in a range of biochemical markers of placental insufficiency and oxidative stress supporting the rationale for prophylactic use of vitamin C and E. Further clinical trials will re-enforce the proof of concept.

The second session started with a lecture from **Dr Irfan Rahman** (Edinburgh, UK), who presented the novel concept of oxidative stress in the regulation of both pro-inflammatory and antioxidant protective genes. Inflammatory lung diseases, such as asthma and chronic obstructive pulmonary disease (COPD), are characterized by systemic and local chronic inflammation and oxidative stress. ROS, either directly or via the formation of lipid peroxidation products such as acrolein, 4-hydroxy-2-nonenal and F_2-isoprostanes may play a role in enhancing the inflammation through the activation of stress kinases (JNK, MAPK, p38, phosphoinositide 3 (PI-3)-kinase/PI-3K-activated serine-threonine kinase Akt) and redox sensitive transcription factors such as NF-κB and AP-1. Interestingly, oxidative stress and pro-inflammatory mediators can alter nuclear histone acetylation/deacetylation allowing access for transcription factor DNA binding leading to enhanced pro-inflammatory (TNF-α, IL-1, IL-8) and protective antioxidant (γ-GCS, MnSOD, HO-1) gene expression in various lung cells. He hypothesized that antioxidant genes are induced during chronic inflammatory responses but this adaptive/protective antioxidant response may be overtaken by pro-inflammatory genes as the inflammation persists, resulting in irreversible lung damage seen in various

chronic lung diseases. Thus, understanding of the molecular mechanisms of ROS-mediated cell signaling pathways would provide information for the development of novel antioxidant therapeutic targets in inflammation and injury.

The therapeutic role of antioxidants from palm oil was described by **Dr Kalanithi Nesaretnam** (Kuala Lumpur, Malaysia) in hormone dependent cancers. She underlined the importance of the specific type of naturally occurring compound, tocotrienols, as one of the new nutritional supplements in reducing the risk of certain chronic diseases including breast cancer. In an interesting animal study, a chemical carcinogen, dimethylbenzathracene-mediated cancer incidence was lowered by feeding palm oil with its vitamin E intact compared to rats fed palm oil stripped of its vitamin E. Tocotrienols were also able to inhibit the proliferation of both estrogen receptor negative and positive human breast cancer cell lines. The result of this study is implicated in human breast cancer and to this effect a clinical trial on primary breast cancer patients is urgently needed.

Professor Junji Yodoi (Kyoto, Japan) presented his ongoing studies on various strategies to provide a proof of concept of novel therapeutic targets for lung injury. Thioredoxin is a redox-active protein and the thioredoxin redox system serves as an important antioxidant and reduction/oxidation (redox) function. The longstanding and compelling work on thioredoxin as a novel antioxidant, a redox sensor and anti-inflammatory compound was confirmed in animal model of pulmonary infection, acute respiratory distress syndrome and pulmonary fibrosis. These researchers have developed a thioredoxin knockout and transgenic mouse to study the mechanism of oxidative stress injury and inflammation. Compared with wild type C57BL/6 mice, thioredoxin overexpressing transgenic mice are more resistant to various oxidative stress associated pulmonary diseases including influenza virus-induced pneumonia, diesel exhaust particle-induced bronchoalveolitis, IL-18/IL-2-induced interstitial pneumonia and bleomycin-induced pulmonary fibrosis. Administration of recombinant thioredoxin attenuates IL-18/IL-2-induced interstitial leukocyte infiltration and prevents lethal lung injury. They have suggested that thioredoxin has a potential for a new therapeutic approach to various chronic inflammatory lung diseases. He also announced that the current exciting studies on "redox sensor" would be discussed in the Redox meeting to be held in Kyoto.

Professor Aldo Tomasi (Modena, Italy) presented data on oxidative stress and inflammation in an LPS model of acute lung injury. In this interesting study, the LPS-mediated oxidative stress and inflammatory response was improved when *N*-acetyl-L-cysteine was administered before intratracheal LPS instillation in rats. This also led to increased GSH levels and a decrease in taurine-chloramine concentration in bronchoalveolar lavage fluid. He also discussed the redox regulation of lung inflammation and emphasized that LPS and NAC may be used in animal model to study the inflammatory response. The mechanism of nitric oxide and carbon monoxide as modulators of synaptic transmission in health and disease was discussed by **Professor Şakire Pöğün** (Izmir, Turkey). A general overview of NO and CO actions on dopamine and glutamate uptake mechanisms was also provided. The next speaker was **Professor Hüveyda Başağa** (Istanbul, Turkey), who presented data on the role of ascorbate on the nuclear binding of AP-1 in macrophages. This study provided insights for the redox regulation of AP-1 and vitamin C was able to amplify the PMA-dependent induction of p38 and JNK kinase pathways. They found that the ascorbate mediated regulation of AP-1 binding was unrelated to free iron but may be regulated by new protein synthesis. In addition, a role for thiols in the regulation of MAPK pathway by ascorbate was proposed.

Professor Tomris Özben (Antalya, Turkey) discussed data on oxidative stress and non-traditional cardiovascular disease risk factors in end-stage renal failure. A role of oxidative and carbonyl stress, oxidized low density lipoprotein, advanced glycation end products, accumulation of asymmetrical dimethylarginine and hyperhomocysteinemia was explored. Homocysteine accumulates in chronic renal patients due to both decreased clearance and impairment of renal metabolic function. Auto-oxidation of homocysteine generates ROS and this pro-oxidant nature of homocysteine causes oxidation of LDL that contributes to atherogenesis. They reported that increased level of plasma homocysteine inhibits glutathione peroxidase activity *in vitro* and decreases endothelial cell mRNA expression of the enzyme. The findings suggest that hyperhomocysteinemia attenuates the antioxidant properties of GSH and thereby potentiates peroxide-mediated cell injury.

Dr Hale Saybaşili (Istanbul, Turkey) presented new data on the effects of selective antagonists and chemical imbalance in superoxide generation in the hippocampus in neurodegenerative disorders. The role of ROS and calcium in pathophysiological functioning of neurons was highlighted. The final talk amongst the list of invited speakers was delivered by **Professor Turgay Dalkara** (Ankara, Turkey). This elegant talk focused on the role of peroxynitrite in reperfusion injury after focal ischemia. NO is generated in the vascular compartment and plays a significant role in reperfusion injury by peroxynitrite formation. The causal relationship between peroxynitrite formation

and vascular damage (Evans blue leakage and MMP-9 expression) was suggested. He concluded that focal ischemia/reperfusion induces superoxide generation and peroxynitrite formation in neurons and astrocytes, as well as the endothelium in the brain. Peroxynitrite mediates reperfusion injury by disrupting vascular integrity.

ORAL PRESENTATIONS

A number of submitted abstracts were also selected for oral presentation. These sessions proved to be a tremendous success because they allowed presentation of novel findings in a number of research fields.

Dr B. Giray (Ankara, Turkey) studied the role of thioredoxin reductase (TR) and antioxidant status in iodine and/or selenium deficient rat livers. It was shown that the iodothyronine deiodinase belongs to a large class of selenoproteins, which catalyzes the metabolic conversion of thyroxine to the most biologically active hormone, 3,5,3′triiodothyronine (T3). They found that the activities of the antioxidant selenoenzymes TR and GSPx were decreased and this was associated with increased lipid peroxidation in selenium and selenium plus iodine deficient rat livers.

Professor El-Naggar (Mansoura, Egypt) described the identification, purification and characterization of 100 kDa protein in sera of mice-treated with a Cu (II) complex with superoxide dismutase-mimetic activity.

Dr B. Sancak (Ankara, Turkey) studied the levels of selenium in serum and ascitic fluid in patients with cirrhosis. They found decreased levels of serum selenium in cirrhotic patients which was not related to the degree of liver cirrhosis and spontaneous bacterial peritonitis.

Professor G. Burçak (Istanbul, Turkey) presented studies of oxidative DNA damage in streptozotocin (STZ)-induced diabetic rats. The levels of 8-hydroxy 2′-deoxyguanonsine (8OhdG) were increased in STZ diabetic rats and the values correlated with glycated hemoglobin values in both diabetic and control rats.

Dr Ç. Yenisey (Aydin, Turkey) reported the levels of proinflammatory cytokines and NO content in pleural effusions. He suggested that pleural inflammation caused by tuberculosis and parapneumonic pleurisy may enhance the release of proinflammatory cytokines and free radicals.

The gene-specific oxidative DNA damage was shown by **Professor M.-H. Chung** (Seoul, Korea) in *Helicobacter pylori*-infected human gastric mucosa. The study revealed that out of the 5 genes tested, p53, insulin like growth factor II receptor and transforming growth factor-β-receptor type II showed significant oxidative DNA damage in *H. pylori*-positive tissues. Prof. Chung concluded that the progressive accumulation of preferential oxidative DNA damage in certain genes such p53, contributes to gastric carcinogenesis.

Dr D. Ivanova (Varna, Bulgaria) measured serum total antioxidant status in myeloblastic and lymphoblastic leukocyte leukemia. It was concluded that uric acid content could be the component that contributed to the very high total antioxidant activity value measured in the course of the whole therapy.

Dr T. Tarcan (Istanbul, Turkey) described hypercholesterolemia and atherosclerosis-induced chronic ischemia by causing structural and functional changes in the rabbit urinary bladder. The mechanism of hypercholesterolemia- and chronic ischemia-induced bladder dysfunction and structural changes remain to be clarified. The expression of heme oxygenase-1 (HO-1) and hypoxia inducible factor-1α (HIF-α) was described under ischemic conditions in human hepatocytes by **Dr E. Müller** (Berlin, Germany). They investigated the effects of cold and warm ischemia on HO-1 mRNA and protein expression in human hepatocytes. This resulted in a time-dependent increase of transcription factor HIF-1α associated with HO-1 induction. The strategies of regulation of HO-1 may be utilized as a therapeutic target in reducing ischemia/reperfusion injury in human liver.

Dr E. Jansen (Bilthoven, The Netherlands) presented the results of studies aimed at improving our understanding of Fenton chemistry in biological system *in vivo*. They used non-transferrin-bound-iron (NTBI) assay on serum samples from a population elderly men from Zutphen (The Netherlands) and correlated with results of oxidative stress markers (hydroperoxides or total antioxidant status) and the iron metabolism. The NTBI parameter showed a positive significant correlation with total iron, the transferring saturation and with serum ferritin. However, there was no correlation with hydroperoxides or the total antioxidant status.

An interesting talk by **Dr M.B. Yerer** (Erciyes, Turkey) claimed that melatonin administration prevents LPS-induced oxidative damage in experimental sepsis in rats. The inhibitory capacity of melatonin was compared with other effective oxidative stress response inhibitor. **Dr Y.A. Yusof** (Kuala Lumpur, Malayasia) presented the results of *Zingiber officinale* (ginger), a traditional medicine used as an anti-inflammatory and antioxidant compound. The active compounds in ginger are gingerol, shogoal, paradol and other phenolic compounds that possess antioxidant properties. They found that the crude extract of *Zingiber officinale* inhibited proliferation and induced apoptosis in a variety of liver cancer cell lines but not in normal cells. It was suggested that crude

extract of *Zingiber officinale* may exert antitumor and antioxidant effects on liver cancer cells.

Dr M. Serteser (Afyon, Turkey) described the gastroprotective effect of epidermal growth factor-mediated induction of antioxidant enzyme activities in the experimental lesions induced by ethanol.

Dr M. Perluigi (Rome, Italy) presented new data on cytotoxicity of tetrahydroisoquinolines on melanoma cells. This is followed by an interesting data presentation by **Dr N. Canacankatan** (Adana, Turkey), who showed that carbosulfan, a carbamate pesticide, depleted GSH associated with increased malondialdehyde in rat liver. Remarkably, administration of carbosulfan histopathologically produced interstitial pneumonia, emphysema, atelectasis and lymphoid hyperplasia in rats. This important observation can be utilized for animal model of emphysema.

Dr J.-W. Park (Taegu, Korea) reported investigations of α-phenyl-*N*-*t*-butylnitrone on the protection of cellular damage induced by ionizing radiation. α-phenyl-*N*-*t*-butylnitrone (PBN), a spin trap, scavenges hydroxyl radicals, protects tissues from oxidative injury. They used 0.1–1 mM PBN to investigate the ionizing radiation-mediated cellular function/damage in U937 cells. PBN protected against lipid peroxidation, oxidative DNA damage and protein oxidation in mouse exposed to whole body radiation. The oxidation of 2,7′-dichlorofluorescein (which measures hydroperoxide production) was decreased by PBN. They concluded that PBN is not only used as a tool for free radical research in EPR but also might have potential in radiation therapy.

FREE RADICAL SCHOOL

The school was organized by **Professor Angelo Azzi** and the first lecture was given by **Professor Aldo Tomasi**. He demonstrated the use of electron spin resonance spectroscopy as a tool for the measurement of free radical species in biomedical studies. **Professor Helmut Sies** gave an excellent overview of profiles of antioxidants in human plasma and accounted the pitfalls of various methods available to measure antioxidant capacity in biological fluids. **Professor Frank Kelly** demonstrated how to study antioxidant biokinetics in human subjects. He started the lecture by demonstrating the possible biokinetics of vitamins C and E by selecting two students from the audience for a practical demonstration. This was followed by **Dr Irfan Rahman's** lecture on measurements of non-invasive oxidant/antioxidant biomarkers in chronic lung diseases. He showed various biochemical and chemical methods and their pitfalls in the measurements of oxidant/antioxidant biomarkers in biological fluids (blood, bronchoalveolar lavage fluid, induced sputum and exhaled breath condensate). Finally, **Dr Tilman Grune** presented two lectures on the measurements of protein oxidation specifically malondialdehyde and 4-hydroxy-2-nonenal. He presented pros and cons of various biochemical marker assays for lipid peroxidation products. The free radical school was a great success not only for researchers new in the field of free radical research but also for investigators who would like to update their knowledge in the context of current technologies and methodologies.

POSTER SESSIONS

Numerous high quality posters were displayed during the conference and these sessions were well attended by both junior and senior investigators. Various aspects of free radical research in health and disease were covered, particularly, studies on naturally occurring compounds, medicinal plants, herbal medicine, fresh leafy vegetables, vitamins, *N*-acetyl-L-cysteine, phenolic compounds, taurine, cod liver oil, and injury healing and repair, inhibition of inflammation, *in vitro* and *in vivo* in animal models and human disease (diabetes, ischaemic heart disease, asthma, chronic obstructive pulmonary disease, renal disease, infertility, epilepsy, bladder obstruction, cancer, preeclampsia, skin disease, multiple sclerosis, atherosclerosis, rheumatoid arthritis, psoriasis, hepatitis, periodontitis, Huntington's disease, hemodialysis, Alzheimer's disease, amebiasis, silicosis, gastrointestinal disease, cancer, schizophrenia and thalassemia). At the same time, the toxicity of various compounds, such as traditional Turkish dental powder, radiation, transition metals (zinc, copper, iron, manganese) were also assessed on cell injury and death.

ROUND TABLE DISCUSSION

Role of Oxidants and Antioxidants in the Regulation of Chronic Diseases: New Research Directions and Applications

This was one of the important parts of the conference, and was chaired by **Professor Lester Packer**. Four eminent scientists in the field of free radical research made up the panel: **Professors Dipak Das**, **Frank Kelly**, **Helmut Sies** and **Junji Yodoi**.

Four areas of discussion emerged as exciting new directions for future research in the field

of free radicals in health and disease. These were: Genonomics/proteomics; Redox signaling; Non-invasive biomarkers and new Methodologies.

The audience was reminded that the functions and roles of 36,000 genes out of 39,000 genes are now known. However, it will take some time to identify the functional responses and expression of these genes (epigenetics) along with genetic polymorphisms in physiology and pathology. Second, the area of redox signaling is very interesting and an improved understanding of the mechanisms of action of redox sensors and biomolecules (low and high doses) will provide the avenue for modulation of biological processes and drug targets. Likewise, the identification and validation of novel non-invasive oxidative stress biomarkers is fascinating. The identification of such biomarkers will be useful in monitoring the oxidative stress in the progress/severity of various diseases and during therapeutic interventions. Finally, new techniques and methods such as analysis of exhaled breath condensate, electron paramagnetic resonance, whole body imaging and real-time laser spectrometer are available to explore redox chemistry.

On the social side, the stimulating talks during the conference were complemented by a fantastic gala dinner held at the Rahmi Koç Industrial Museum. Participants benefited from the picturesque scene of sunset over the Golden Horn. The dinner with Raki (Turkish drink) set the scene for the evening of a gathering of free radical scientists came from every corner of the world. The attendees gave full appreciation and applause to **Professors Nesrin Kartal Özer** and **A. Süha Yalçin** for their painstaking effort to make the conference an amazing success.

Please visit the SFRR website for more information and a preliminary program for the SFRR (International) meeting to be held in Paris, July 16–20, 2002; and the next European SFRR meeting: European Section on 26–29th June, 2003, Ioannina, Greece: http://www.sfrr.com or http://www.conferre.gr or http://www.uoi.gr/conf_sem/sfrr or email E-mail: info@conferre.gr.