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Meeting Report

The IX Biennial Meeting of the International Society for Free Radical Research, Sao Paulo, Brazil, September 7–11, 1998

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OPENING REMARKS

The IX Biennial Meeting of the ISFRR started with opening remarks by Dr. Ohara Augusto, who pointed out that this was the first meeting of the ISFRR in Latin America and it was attended by a great number of participants from Latin America. Consequently, this provided a unique opportunity for scientists from this region to strengthen bonds of collaboration between themselves and their colleagues from all over the world. Dr. Augusto also stated that one of the main aims of this meeting was to improve human health.

Dr. Helmut Sies outlined the multidisciplinary activity of the ISFRR as well as the considerable growth of this Society. He welcomed young researchers and emphasized the importance of stimulation of future research.

BIOLOGICAL SOURCES AND TARGETS OF ELECTRONICALLY EXCITED STATES

Dr. W. Adam reported a high photooxidative DNA-damaging efficiency of the most reactive dioxetane, the hydroxymethyl-trimethyl derivative (HTMD), due to radical activity. Indeed, HTMD gave high yields of 8-oxodeoxyguanosine and significant amounts of guanidine-releasing products oxazolone and oximidazolidine as well as 4-hydroxy-8-oxodeoxyguanosine. Thus, he suggested that DNA oxidation by radicals is much more effective than that of triplet excited-ketones.

Dr. E.A. Lissi reported that a high proportion of the chemiluminescence (CL) produced in the visible range is not due to the occurrence of biomolecular processes between chain carrying radicals but is associated with the accumulation of oxidation products. The production of light from these intermediates is not a simple unimolecular process and appears to be mediated by free radical reactions. Dr. Lissi also compared the CL associated with oxidation of biological membranes with that of lipid and protein oxidation.

Dr. C.A.A. Penatti reported that peroxynitrite catalyzes the aerobic oxidation of isobutanal with generation of electronically excited triplet acetone. Dr. Penatti postulated that triplet acetone

results from the cyclization of an alkylperoxyl radical to a dioxetane radical intermediate, followed by its thermolysis.

Dr. W. Hansberg reported that singlet oxygen modifies catalase from *Neurospora crassa*, giving rise to a shift in electrophoretic mobility similar to the one observed *in vivo* during development and under stress conditions. Furthermore, bacterial, fungal, plant and animal catalases were susceptible to modification by singlet oxygen. Hence, modification of catalases during development and under stress could indicate *in vivo* generation of singlet oxygen.

Dr. E.A. Prieto reported that carcinine (β-alanyl-histamine), a powerful antioxidant which protects deoxyribose against damage by $OH^{\bullet}$ reduced markedly the extent of apoptosis induced by rUVA in human keratinocytes NCTC 2544, as evidenced by flow cytometry analysis with propidium iodide and a decrease in the amount of oligonucleosomes.

FREE RADICAL REACTIONS AND BIOMOLECULAR DAMAGE

Dr. C.C. Winterbourn reported that HOCl is a strong oxidant towards a wide range of biological molecules, particularly proteins and glutathione, and is highly cytotoxic. However, cells treated with very low (μM) doses of HOCl undergo more specific functional changes, such as inhibition of glyceraldehyde 3-phosphate dehydrogenase, growth arrest, p53 activation and apoptosis. Thus, hypochlorous acid acts not only as a toxic agent but also as mediator in the inflammatory response.

Dr. W.H. Koppenol showed that nitrogen monoxide is responsible for the formation of nitrosothiols, peroxynitrite as well as nitrosyl complexes with haem proteins. Peroxynitrite may react with biomolecules and yield NO_2^- which, in turn, may react with HOCl to form nitryl chloride. Furthermore, formation of peroxynitrite cannot be prevented by superoxide dismutase near activated macrophages, where $O_2^{\bullet -}$ and $NO^{\bullet}$ react at micromolar concentrations.

Dr. S. Fu reported that a significant amount of 3,5-dichlorotyrosine was obtained from BSA treated with hypochlorite. Thus, he suggested that 3-chloro-tyrosine (3-Cl-Tyr) has relatively higher reactivity towards HOCl than tyrosine itself. These results indicate the importance of assessing products of HOCl action in addition to 3-Cl-Tyr.

Dr. A.W. Girotti showed that there is a direct correlation between Se availability, GSH/selenoperoxidase activity and cell ability to detoxify and thereby tolerate lipid hydroperoxides. Thus, reduction of lipid hydroperoxides by GSH/selenoperoxidase can contribute to tumor resistance in photodynamic therapy.

Dr. M. Takahashi reported that apoptosis induced by hydroperoxides and a lipid-soluble free radical initiator was effectively suppressed in vitamin E-enriched U937 cells. However, a marginal effect of vitamin E was observed with other apoptosis-inducing stimuli, when pretreatment with intracellular water-soluble radical scavengers was effective. Hence, he suggested that lipid peroxidation initiates apoptosis only when it induces cell injury as primary damaging species.

DNA DAMAGE AND REPAIR

Dr. J. Cadet pointed out that most of the assays of oxidized bases within cellular DNA suffer from major drawbacks which lead to an overestimation of the level of the targeted molecule. One exception has been the accurate measurement of 8-oxo-7,8-dihydroguanine by HPLC with electrochemical detection (ECD). Significant improvement has also been recently achieved with the development of accurate HPLC/GC–MS as well as HPLC-^{32}P-postlabelling methods together with a modified Comet assay involving the use of DNA repair glycosylases.

Dr. M. Dizdaroglu reported that DNA glycosylases are specific for either pyrimidine or purine lesions or both. Furthermore, these enzymes were not able to excise completely all modified bases and kinetic constants depended on the nature of DNA substrates and varied among base lesions for the same substrate.

Dr. S. Loft and coworkers studied 8-oxodeoxyguanosine and 8-aminoguanosine levels in tissues from rats treated with different inducers of oxidative DNA damage, such as 2-nitropropane, iron dextran or lipopolysaccharide. Dr. Loft reported that the type of treatment determines the major modified nucleotide as well as the tissues which suffer from oxidative damage.

Dr. E.S. Henle reported the preferential cleavage sites of duplex DNA by Fe^{2+} and low or high concentrations of H_2O_2. He also showed that telomere inserts were specially sensitive to oxidative damage.

Dr. V.I.M. Carvalho showed that trans,trans-2,4-decadienal (DDE), an important breakdown product of lipid peroxidation, can be epoxidized by peroxides and the resulting products are able to form adducts with 2′-deoxyadenosine and or/DNA. Thus, he suggested that endogenous DNA adduct formation may contribute to the high cytotoxicity of DDE in mammalian cells.

FREE RADICAL DETECTION AND BIOMARKERS

Dr. B. Kalyanaraman reported that superoxide generation by endothelial nitric oxide synthase (eNOS) is enhanced by flavins, but abolished by concomitant addition of L-arginine and tetrahydrobiopterin (BH4). Moreover, addition of redox agents, such as doxorubicin, enhanced superoxide formation by eNOS.

Dr. R.P. Mason showed data supporting the view that tyrosine nitration by the reaction of tyrosyl radical with nitric oxide and/or nitrogen dioxide radicals occurs without involving peroxynitrite.

Dr. M.J. Davies reported that 3-hydroxylysine and 4-hydroxylysine may be sensitive markers of radical-mediated protein oxidation. Furthermore, Dr. Davies stated that surface protein residues, such as lysine, give higher yields of oxidized products than do hydrophobic residues, such as leucine and valine.

Dr. S. Hix reported that tert-butyl hydroperoxide appears to be metabolized to methyl radicals and it is able to alkylate DNA *in vivo*. Hence, DNA methylation may be involved in cytotoxicity induced by organic peroxides and hydroperoxides.

Dr. H. Sano and coworkers studied new spin-probes to measure free radical reactions in the brains of living animals. Dr. Sano reported that carboxy-PROXYL acetoxymethyl ester is a good candidate for that, because it is metabolized specifically by brain esterase into hydrophilic metabolites, resulting in a long-term retention in the brain of mice.

REDOX REGULATION OF CELL SIGNALLING AND GENE EXPRESSION

Dr. A. Azzi pointed out that certain physiological antioxidants, such as α-tocopherol, may exhibit other actions in addition to their antioxidant capacity and hence, they may modulate signal transduction pathways through mechanisms not related to their antioxidant properties. Thus, he reported that α-tocopherol, but not β-tocopherol, may reduce the proliferation of vascular smooth muscle cells by inhibiting protein kinase C (PKC). This inhibition is due to PKC-α dephosphorylation caused by activation of protein phosphatase 2A.

Dr. M. Karin reported that glutathione depletion is, at least in part, involved in NF-κB activation by UV, but it does not seem to be involved in NF-κB activation by γ-radiation. Consequently, he suggested that oxidative intermediates are unlikely to participate in activation of NF-κB or IκB kinase by γ-radiation, although

they may play a partial role in the IκB kinase independent UV activation pathway.

Dr. T. Galeotti reported an inverse correlation between Mn-superoxide dismutase (MnSOD) expression and the functional status of p53 in a number of tumor cell lines as well as an increase in MnSOD content in tissues from p53 deficient mice. In addition, overexpression of MnSOD in Hela cells appears to confer resistance to growth arrest and/or apoptosis triggered by overexpression of p53. Hence, Dr. Galeotti suggested that MnSOD repression is essential for p53 induced cell death.

Dr. J.S. Woods showed that activation of NF-κB in renal cells is not responsive to oxidative stress, but its activation by lipopolysaccharide is modulated by intracellular calcium levels. These results suggest a tissue-specific expression and function of NF-κB in kidney epithelial cells.

Dr. S.S. Chan reported that α-tocopherol may modulate tyrosine phosphorylation-dependent signalling processes related to the oxidative burst in neutrophils. Furthermore, this modulation was dependent on the nature of the stimulus given to the cells.

Dr. C. Pasquier reported that reactive oxygen species (ROS) induce the adhesion of neutrophils to endothelial cells by activation of tyrosine kinases, such as $p125^{FAK}$. This ROS-induced adhesion is prevented by antioxidant agents, such as the *Ginkgo biloba* extract EGb 761, which may be useful to protect against chronic inflammation.

HEME AND IRON METABOLISM

Dr. R. Meneghini pointed out that cellular DNA damage under pro-oxidant conditions is mediated by iron through a Fenton-type reaction. Dr. Meneghini reported that iron is present in the nucleus, as evidenced by EM elemental analysis. Furthermore, a P-type ATPase is responsible for transport of iron into the nucleus.

Dr. M.W. Hentze commented that iron metabolism is controlled post-transcriptionally by two cytoplasmic regulatory proteins: iron regulatory protein 1 (IRP-1) and IRP-2. The IRPs mediate translational repression of ferritin and erythroid 5-aminolevulinate synthase expression, whereas they convey stabilization of transferrin receptor mRNA. IRP-1 activity is controlled by cellular iron levels as well as by signalling through NO and reactive oxygen intermediates. IRP-2 is regulated only in response to iron levels and NO.

Dr. M. Comporti reported that iron release is a factor in generation of senescent cell antigen (SCA), which is a signal for termination of old erythrocytes by initiating the binding of autologous IgG and subsequent removal by phagocytes. Dr. Comporti and coworkers found that there is iron release together with SCA generation during *in vitro* aging of erythrocytes and this SCA generation can be prevented by iron chelators.

Dr. J.M. De Freitas reported that the low affinity iron uptake protein Fet4p was involved in the elevation of iron content found in the *Saccharomyces cerevisiae* mutant lacking Cu/Zn-superoxide dismutase. Thus, he proposed that the increased iron content in that mutant may be a reflection of cellular efforts to reconstitute [4Fe-4S] cluster-containing enzymes that have been inactivated by excess superoxide.

Dr. G.R. Braz showed that oogenesis in *Rhodnius prolixus* females is dependent on the operation of their heme biosynthetic pathway. He reported that oviposition was strongly inhibited by the addition of succinil acetone, a known specific inhibitor of ∂-aminolevulinate dehydratase (ALA-D) - the second enzyme of the heme biosynthetic pathway. Moreover, normal oviposition was restored if females were fed with the product of the ALA-D reaction.

Dr. K.S.A. Mossanda reported that phototherapy treatment significantly decreased the levels of bilirubin and malondialdehyde as well as increased the trolox equivalent antioxidant capacity in non-asphyxia term babies. However, phototherapy treatment did not change significantly bilirubin levels nor did it improve the

antioxidant status of the premature babies, especially those with asphyxia.

REGULATION OF NO PRODUCTION

Dr. S.S. Gross showed that GTP cyclohydrolase I (GTPCH) and argininosuccinate synthetase (AS) play a key role, in addition to iNOS gene expression, in high-output NO synthesis in vascular smooth muscle cells. GTPCH is rate-limiting for *de novo* synthesis of tetrahydrobiopterin, a required cofactor of NOS, whereas AS contributes to recycle arginine from the NOS co-product, citrulline.

Dr. A. Tomasi and coworkers found a marked elevation in NO derived from a NOS-independent pathway in rat skeletal muscle subjected to ischemia-reperfusion injury. Indeed, inhibitors of NOS did not prevent the increase in levels of muscle nitrosoheme complexes found after ischemia-reperfusion.

Dr. S. Zöllner showed that the expression of endothelial NO synthase is proliferation-dependent in bovine atrial endothelial cells. Dr. Zöllner reported that endothelial NO synthase is up-regulated in proliferating cells and down-regulated in quiescent cells. Nevertheless, superoxide generation by highly proliferating endothelial cells decreases bioavailable NO in these cells.

Dr. C.M. Arroyo showed data supporting the role of interleukin 1β (IL-1β) and tumor necrosis factor alpha (TNF-α) in epidermal cytotoxicity induced by sulfur mustard. Thus, Dr. Arroyo reported that sulfur mustard induces an increase in IL-1β levels in adult human breast epidermal keratinocytes as well as in TNF-α levels in human monocytes.

Dr. T.M. Millar and coworkers demonstrated that xanthine oxidase is able to reduce nitrates and nitrites to nitric oxide using NADH as an electron donor. Thus, Dr. Millar suggested a role for xanthine oxidase in NO generation particularly under hypoxic conditions, which is when NO synthase cannot function.

PATHOLOGICAL IMPLICATIONS OF NO

Dr. R. Radi showed that peroxynitrite ($ONOO^-$) is formed from the fast combination reaction between NO and superoxide radicals. $ONOO^-$ crosses cell membranes either by anion channels or passive diffusion and it contributes to cell and tissue injury through a variety of mechanisms including oxidation and nitration of critical targets. Nitration reactions are catalyzed *in vivo* either by transition metal centers or by CO_2. Dr. Radi commented that $ONOO^-$ tiggers cell death both via apoptotic and necrotic mechanisms.

Dr. H. Sies reported that glutathione peroxidases (GPx) exhibit peroxynitrite reductase activity using GSH as a reductant. Thus, GPx protect against protein 3-nitrotyrosine formation and against the oxidation of dihydrorhodamine 123 by peroxynitrite. Hence, GPx have a role in GSH-dependent maintenance of a defense line against peroxynitrite-mediated reactions.

Dr. H. Nakazawa showed that tetrahydrobiopterin (BH4) levels increased markedly in shock patients and in endotoxin-treated animals, but this increase was reduced significantly by polymyxin B (PMX) hemoperfusion. These data support the view that the removal of BH4, an essential co-factor of NO synthase, is responsible for the recovery of blood pressure by PMX.

Dr. P. Moriel reported that α-tocopherol, lycopene and β-carotene levels were lower in plasma of hypercholesterolemic and/or hypertensive subjects than in plasma of normo-lipidemic normotensive subjects. Consequently, Dr. Moriel suggested that the impairment of endothelium-dependent relaxation in hypertensive and hypercholesterolemic patients may be related to a lower plasma antioxidant content.

Dr. A. Denicola reported that CO_2 at physiological concentrations prevents only partially (less than 30%) the oxidation of intracellular hemoglobin by extracellular peroxynitrite. Hence, peroxynitrite is able to reach the inside of erythrocytes and oxidize intracellular proteins even in the presence of CO_2.

Dr. C.E. Robinson reported that levels of 4-hydroxynonenal in colonic mucosa were elevated in both Crohn's disease and ulcerative colitis, whereas nitrotyrosine levels were higher in Crohn's disease than in ulcerative colitis or controls. Thus, colonic mucosa suffers oxidative damage in both Crohn's disease and ulcerative colitis. Furthermore, nitrotyrosine appears to be a more significant product in Crohn's disease as compared to ulcerative colitis.

XENOBIOTICS AND OXIDATIVE STRESS

Dr. L.A. Videla showed that lindane induces oxidative stress in the liver by two different mechanisms, one early component independent of cytochrome P450 induction and another late component associated with P450 induction, which involves higher rates of superoxide generation and lipid peroxidation.

Dr. M.U. Dianzani showed that 4-hydroxynonenal may act as a signal for certain cell functions as well as in the cell cycle. This aldehyde is provided with chemotactic–chemokinetic properties towards polymorphonuclear leukocytes. Moreover, this aldehyde blocks the expression of the oncogene c-myc and decreases cell proliferation in different leukemic human cell lines.

Dr. L.S. Nakao showed data supporting a role for carbon-centered radicals in alcohol toxicity. Dr. Nakao demonstrated that 2-hydroxyethyl-radical and methyl radicals are formed, in addition to acetaldehyde and the 1-hydroxyethyl radical, during ethanol oxidation by H_2O_2/Fe(II) and peroxynitrite.

Dr. T. Wakabayasi showed that apoptotic changes in cells were found after long treatment (for 72 h) with free radical inducer agents which produce megamitochondria. These data support a role of megamitochondria in the induction of apoptosis.

Dr. H. Alho and coworkers found that urinary excretions of 8-hydroxydeoxyguanosine in moderate or heavy alcohol drinkers were not elevated when compared with that of healthy volunteers. Furthermore, oxidized LDL and total antioxidant capacity of LDL were not significantly different among these groups. Thus, Dr. Alho suggested that a systemic oxidative stress is not associated with alcohol consumption.

OXIDATIVE STRESS AND PATHOGENIC MICROORGANISMS

Dr. P.R. Ortiz de Montellano commented that isoniazid resistance in *M. tuberculosis* has been shown to involve suppression of katG, a peroxidase that oxidizes isoniazid to a reactive species that inhibits cell wall biosynthesis. Dr. Ortiz de Montellano reported that the loss of katG appears to be compensated for by an increase in AhpC, a putative non-heme peroxidase. Therefore, AhpC or other peroxidase proteins may also be suitable targets for the development of anti-tuberculosis agents.

Dr. L. Flohé showed that hydroperoxide removal in trypanosomatids is catalyzed by a unique cascade of oxidoreductases: the flavoprotein trypanothione reductase, the thioredoxin-related tryparedoxins and the peroxiredoxin-type tryparedoxin peroxidase. Dr. Flohé pointed out the potential use of these enzymes as targets for the design of trypanocidal drugs.

Dr. J.J. Maguire reported that monochloramines, which are produced by neutrophils during the respiratory burst, causes a rapid turnover of glutathione and are potent inhibitors of PKC. Consequently, monochloramines may act as one of the signals from the activated respiratory burst to adjacent cells and they may modulate activity of PKC in adjacent cells.

Dr. K. Kasazaki reported an enhancement of radical generation in ammonia-induced gastric ulcer in the rat by using the *in vivo* ESR technique with the nitroxide probe 3-carbamoyl PROXYL. However, Dr. Kasazaki showed that monochloramine-induced gastric ulcer follows a different mechanism since it is not associated with

radical generation nor with myeloperoxidase activity.

Dr. J. Fuchs reported that clastogenic factors (CF) appear very early in HIV infection and they correlate negatively with CD4+ T-cells. However, CF are not specific for the HIV-1 infection, because they also occur in HIV-1 negative patients with malignant tumors.

PRO-OXIDANTS IN FOOD

Dr. A. Sevanian reported that plasma lipid peroxides are elevated in the postprandial state and this may contribute to the correlation between postprandial hyperlipidemia and increased risk of cardiovascular disease. The levels of postprandial peroxides are influenced by dietary antioxidants and the antioxidant capacity of other food components. Moreover, the individual capacity for reduction of peroxides in the intestine influences the fate of ingested peroxides.

Dr. Sevanian also showed that cholesterol oxidation products inhibited caveolin translocation to the plasma membrane and efflux of LDL-derived or *de novo* synthesized free cholesterol in endothelial cells. Thus, Dr. Sevanian postulated that inhibition of caveolin-mediated free cholesterol translocation by cholesterol oxidation products contributes to free cholesterol accumulation.

Dr. F. Ursini confirmed that the major plasma antioxidant capacity is accounted for by ascorbate, urate and albumin thiol. Furthermore, usual nutritional doses of polyphenols markedly increased the plasma antioxidant capacity and also lowered the postprandial increase in plasma lipid hydroperoxides.

Dr. A.F.P. Moura and coworkers found a relatively high concentration of 7-ketocholesterol, an indicator of cholesterol oxidation, in fresh shrimp-pink, likely due to inappropriate storage conditions. Dr. Moura concluded that 7-ketocholesterol could be used as an indicator of fresh shrimp-pink adulteration during storage on board and commercialization.

Dr. E.C. Pincinato and coworkers studied the esterification by lecithin cholesterol acyl transferase (LCAT) of cholesterol and cholesterol oxides incorporated into HDL. Dr. Pincinato reported that the higher the cholesterol oxide concentration the lower was the esterification by LCAT. These results suggest that cholesterol oxide-enriched HDL is a less efficient substrate for LCAT and that cholesterol oxides inhibit LCAT activity.

LDL OXIDATION AND ATHEROSCLEROSIS

Dr. D. Steinberg outlined the important role of LDL oxidation in atherogenesis. He commented on the mechanisms involved in LDL oxidation, the role of scavenger receptors of macrophages in generation of foam cells as well as the current status of clinical intervention trials.

Dr. B. Frei pointed out that vitamin C inhibits LDL oxidation and reduces *in vivo* levels of F2-isoprostanes, which are end products of lipid peroxidation. Dr. Frei also reported that vitamin C restores the biological activity of NO which is impaired in cardiovascular disease (CVD) patients and hence, may exert beneficial effects on CVD by preventing dysfunction of the vascular endothelium.

Dr. R. Stocker pointed out that although several intervention studies have found that certain antioxidants can inhibit atherosclerosis, antioxidants do not always inhibit it in animals, and when they do, the underlying action is often not verified. Hence, further research is needed to assess the temporal relationship between LDL oxidation and atherogenesis and how these processes compare with each other in the different animal models used for antioxidant intervention studies.

Dr. J.K. Keaney reported that administration of water- and lipid-soluble antioxidants improves nitric oxide-mediated vascular funcion. This effect appears unrelated to inhibition of LDL oxidation. Thus, Dr. Keaney outlined the direct

vascular effects of antioxidants on nitric oxide-mediated vascular function.

Dr. H.P. Souza showed that a marked increase in oxygen free radical generation occurs early after vessel injury. This increase was amplified in the presence of exogenous NADH/NADPH, inhibited by a flavoenzyme inhibitor and not related to xanthine oxidase, NO synthase or mitochondrial electron transport. Thus, Dr. Souza concluded that arterial injury induces early activation of medial/adventitial NAD(P)H oxidase(s) producing radical species potentially involved in tissue repair.

Dr. R. Mashima reported that two hydroperoxide-reducing protein fractions were isolated from human plasma and one of the proteins was identified as apolipoprotein A-I. Dr. Mashima also demonstrated that methionines of apolipoprotein A-I reduce lipid hydroperoxides to their hydroxides with concomitant oxidation of methionine to methionine sulfoxide.

Dr. P.K. Witting reported that there is a dissociation of intimal lipid peroxidation and atherogenesis in WHHL rabbits, which indicates that lipoprotein lipid peroxidation is not required for early stages of atherosclerosis development in this experimental model. Thus, Dr. Witting showed that the antiatherogenic effects of probucol in WHHL rabbits may not be due to inhibition of lipoprotein lipid peroxidation.

NEURODEGENERATIVE DISEASES

Dr. M.F. Beal commented on the experimental evidence which supports the involvement of oxidative damage in the pathogenesis of neurodegenerative diseases such as Friedrich's ataxia, amyotrophic lateral sclerosis, Huntington's disease and Alzheimer's disease. Dr. Beal outlined the role of a defect in transcription of a mitochondrial protein, which regulates iron transport, in the pathogenesis of Friedrich's ataxia.

Dr. L.R. Ramos spoke about the experimental evidence in favor of the role of oxidative damage in Alzheimer's dementia. He reported that there is a tendency for correlation between low plasma levels of antioxidants, higher oxidative stress and less functional capacity, especially cognitive impairment.

Dr. M.M.M. Grazina showed a correlation of mitochondrial function with serotonin receptor HTR2A genotypes in Alzheimer's disease patients. This correlation was dependent on the age of patients. Thus, Dr. Grazina suggested a role of mitochondria in neurodegeneration.

Dr. T. Orbed showed that oxidative stress occurs during cerebral ischemia/reperfusion. Furthermore, Dr. Orbed reported that inhibition of glutamate release by lamotrigine increased SOD and glutathione peroxidase levels, especially in cortical and cerebellar regions during cerebral ischemia/reperfusion.

Dr. A.G. Estévez reported that SOD becomes cytotoxic when it is Zn-deficient. Moreover, SOD toxicity requires the copper in the active site. Dr. Estévez also showed that induction of motor neuron apoptosis by Zn-deficient SOD was mediated, at least in part, by peroxynitrite.

OTHER PRESENTATIONS OF MEDICAL INTEREST

Dr. L. Montaigner pointed out the involvement of oxidative stress in HIV-induced lymphocyte dysfunction and death. Dr. Montaigner reported that key proteins from lymphocytes of AIDS patients are highly oxidized, resulting in a faster degradation by proteolytic enzymes. Thus, the shortage of IL2 as well as the impairment of lymphocyte functions may contribute to immune suppression. Hence, a treatment against oxidative stress should be considered in addition to antiretroviral therapy.

Dr. D. Ziegler showed that oxidative stress is involved in the development of diabetic

complications. Thus, impaired endothelium-dependent vasodilatation is improved by administration of vitamin C. Moreover, treatment with a variety of antioxidant compounds prevents or reverses the deficits associated with diabetic neuropathy and high doses of vitamin E can reverse the hemodynamic changes observed in early stages of diabetic retinopathy and nephropathy.

Dr. G. Poli showed data supporting a correlation between oxidative stress and cytolysis markers early after reperfusion of implanted liver. He reported that patients receiving a multivitamin infusion showed a slower increase of serum transaminases as well as a delayed neutrophil activation soon after liver reperfusion.

NUTRITION, ANTIOXIDANTS AND HEALTH

Dr. L. Packer showed that free radicals at low levels are signalling molecules, whereas when overproduced are cytotoxic. Hence, antioxidants should modulate gene expression, slow aging and disease. Dr. Packer commented on epidemiological studies which correlated antioxidant intake and the relative risk of disease. He also pointed out that vitamin C is the hub of the antioxidant network. He suggested that flavonoids and polyphenols may possibly act as the interface between ascorbate and lipophilic antioxidants, such as vitamin E. Dr. Packer also reported that flavonoid-containing mixtures may modulate nitric oxide metabolism and cell adhesion molecules.

Dr. J. Blumberg spoke of antioxidants in health promotion, focusing on the impact on disease incidence and health costs. He pointed out the importance of antioxidant consumption and supplementation for health promotion and disease prevention.

Dr. E. Niki pointed out the factors considered in designing radical-scavenging antioxidants. These factors are: the structure–reactivity correlation to maximize the reactivity of the compound towards radicals; the stability and fate of the antioxidant-derived radical; the concentration, location and mobility at the microenvironment; interactions with other antioxidants and toxicity. Dr. Niki and coworkers have designed a novel antioxidant, 2,3-dihydro-4,6-dimethyl-2,2-dipentyl-5-hydroxybenzofuran, which appears to be more potent than tocopherol and a promising candidate as an antioxidant drug.

Dr. T.M. Bray pointed out the role of oxygen free radicals in childhood diseases such as bronchopulmonary dysplasia, cerebral palsy and type 1 diabetes. Dr. Bray studied the role of Zn, vitamin E and glutathione in the susceptibility to hyperoxia-induced oxidative damage in lung and brain of the young. Moreover, Dr. Bray used transgenic mice to assess the role of CuZnSOD in protection against type 1 diabetes.

Dr. R. Brigelius-Flohé reported that mRNA levels of gastrointestinal glutathione peroxidase were increased in selenium deficiency due to an increase in mRNA stability. These results support the role of this enzyme as a primary barrier against alimentary hydroperoxides.

Dr. R.J. Ulvik and coworkers found a decrease in the antioxidant capacity both in the intestinal mucosa and in blood in chronic inflammatory bowel disease. Furthermore, iron therapy increased the consumption of antioxidants and hence, antioxidant treatment in that disease should be considered.

Dr. H. Lenhartz showed that antioxidant supplementation with ascorbate, α-tocopherol and N-acetyl cysteine exhibited beneficial effects in the treatment of Kwashiorkor syndrome, as evidenced by an increase in the daily loss of oedema. Since the mortality in oedematous malnutrition correlates with the severity and persistence of oedema, Dr. Lenhartz pointed out the promising effects of antioxidant supplementation.

Dr. R. Garay showed that EGb 761, a *Ginkgo biloba* extract with antioxidant properties, protects against ileo-femoral artery restenosis in the rabbit.

Indeed, vascular lessions assessed by histological analysis were less frequent and with less size and tightness in EGb 761-treated rabbits.

AGING: FROM MOLECULAR MECHANISMS TO THERAPEUTIC INTERVENTION

Dr. D. Harman spoke of the free radical theory of aging (FRTA), which postulates that aging changes are produced by free radical reactions. Changes produced by these reactions can be decreased by lowering initiation rates and/or chain lengths. Application of the FRTA has been fruitful since antioxidant administration may increase average life expectancy and it provides insight into the pathogenesis, prevention and treatment of age-related diseases.

Dr. K.J.A. Davies pointed out the possibility that cellular shock and stress genes and proteins may lose upon aging their ability to respond rapidly and adequately to transient changes in oxidative stress levels. Thus, he suggested that future research may involve gene therapies to activate endogenous damage removal and repair systems as well as shock or stress genes.

Dr. T. Grune reported that oxidized proteins accumulate during proliferative senescence of human fibroblasts, likely due to a decrease in the protein turnover. Furthermore, Dr. Grune showed that the ability of cells to degrade hydrogen peroxide-oxidized proteins decreased in proliferative senescence.

Dr. C. Leeuwenburgh showed that determinations of oxidized amino acids, such as *o,o'*-dityrosine and *o*-tyrosine, in urine by GC/MS serve as a non-invasive measure of oxidative stress *in vivo*. Indeed, urinary levels of these oxidized amino acids mirrored those of skeletal muscle during acute exercise and aging in rats. Moreover, urine levels also increased in humans with age or in Kwashiorkor syndrome.

Dr. N.C. Souza-Pinto and coworkers measured the activities of mitochondrial oxidative damage endonuclease and mitochondrial uracil DNA glycosylase in rat liver and heart mitochondria upon aging. They found an increase in oxidative DNA damage repair in mitochondria with age. Thus, Dr. Souza-Pinto suggested that this increase might be due to the accumulation of lesions with age.

Dr. J.Y. Choi and coworkers identified a novel point mutation in the 8-hydroxyguanine glycosylase (ogg1) gene in the senescence-prone strain (SAMP) of senescence accelerated mice, which may be responsible for the reduced ogg1 activity as well as the increase in 8-hydroxydeoxyguanosine (8OHdG) content in DNA found in organs of SAMP mice. Thus, Dr. Choi suggested that the impairment of 8OHdG repair in SAMP mice may be one of the factors contributing to accelerated senescence.

FREE RADICALS AND MITOCHONDRIAL DAMAGE

Dr. Alberto Boveris gave the opening lecture on mitochondrial aging. Dr. Boveris said that mitochondria have a prominent role in the free radical theory of aging because they are the main source of intracellular prooxidant species, they suffer oxidative damage with age and they are able to signal for apoptosis. Indeed, changes in mitochondrial function and morphology have already been reported in cellular aging. Recently, Boveris and coworkers discovered at the same time as Richter and coworkers that mitochondria produce NO and they contain NO synthase activity. NO may increase superoxide production by mitochondria and, thus, it may be involved in mitochondrial aging.

Dr. E. Cadenas showed that the respiratory control and the formation of pro-oxidant species at the mitochondrial respiratory chain are modulated by the concentration of nitric oxide. In addition, he found that glutathione depletion in mitochondria is associated with less oxidative damage to mitochondrial DNA and less single strand breaks.

Dr. A. Vercesi showed that reactive oxygen species play a pivotal role in generation of the mitochondrial permeability transition (MPT). Thus, MPT is induced by pro-oxidants and inhibited by antioxidants.

Dr. J. Bustamante showed that decreases in respiratory control and mitochondrial glutathione content occur as early events during rat thymocyte induced apoptosis.

Dr. W. Augustin showed that hypoxia/reoxygenation in isolated mitochondria is associated with impaired oxidative phosphorylation and oxidative stress, which was evidenced by oxidative damage to mitochondrial lipids, proteins and DNA.

Dr. F. Sluze showed that phosphorylating respiration is impaired and ADP/O ratio is decreased in mitochondria after ischemia-reperfusion *in vivo* as well as after anoxia-reoxygenation *in vitro*. He also found that the *Ginkgo biloba* extract EGb 761 has a protective effect on these respiratory parameters.