ANTIOXIDANTS & REDOX SIGNALING
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Conference Report

Third International Conference on "Oxygen/Nitrogen Radicals: Cell Injury and Disease" Morgantown, West Virginia, June 1–5, 2002

THIS MEETING was dedicated to the molecular mechanisms of disease development and emerging strategies for prevention and treatment of various diseases. The primary educational objective of the conference was to provide a forum to discuss state-of-the-art knowledge on the role of reactive oxygen/nitrogen species (RONS) in the development of Alzheimer's disease, atherosclerosis, diabetes, disorders of the eye, nutritional disorders, Parkinson's disease, pulmonary disorders, rheumatoid arthritis, and skin diseases. Lectures covering various molecular aspects of disease development and strategies for intervention were presented by 44 invited international experts and were discussed by the conference participants. Platform presentations were published in *Molecular and Cellular Biochemistry,* Volume 234/235, May/June 2002. In addition to the invited lectures, 109 abstracts were presented at a poster session and discussed by the attendees.

The opening remarks were presented by the chairperson, Dr. Val Vallyathan, Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, Morgantown, WV. He briefly reviewed the significant progress that has been made during the last few years in defining the exact molecular mechanisms involved in the development of certain diseases and called attention to the challenging work lying ahead to build a pipeline from laboratory research to the clinic for the development of therapeutic intervention of diseases caused by RONS.

Dr. Bruce Demple from the Harvard School of Public Health in Boston, MA, presented a plenary talk describing the dramatically different modes of nitric oxide (NO) signaling and regulating cellular functions. The transduction of NO signals through superoxide regulon (SoxR) was shown to have a significant influence on gene activation mediated by SoxR. The presence of this signaling pathway was shown to be important in harnessing cellular stress. Adaptive NO resistance was also shown to play an important role in the induction of heme oxygenase-l, DNA repair, and protein turnover.

Dr. Earl Stadtman from the National Institutes of Health, Bethesda, MD, presented evidence that all forms of reactive oxygen species (ROS) oxidize methionine residues of proteins to a mixture of the *R*- and *S*-isomers of methionine sulfoxide. The cyclic interconversion of protein methionine residues between oxidized and reduced forms can scavenge ROS and thus may have an important role in cellular regulation. Methionine sulfoxide reductases can regulate the lifespan of organisms either under normal conditions or when under oxidative stress.

Dr. Chevion from the Hebrew University–Hadassah, Jerusalem, Israel, provided a report on the protection of the heart from ischemia/reperfusion injury by preconditioning the heart with three episodes of 2 min of ischemia separated by 3 min of reperfusion. Findings show that intracellular redistribution and mobilization of small levels of iron during preconditioning causes rapid accumulation of ferritin, the major iron-storage protein, and plays a dual role in the signaling pathway for the accumulation of ferritin following the preconditioning phase.

Dr. Balasubramanian from the L.V. Prasad Eye Research Institute in Hyderabad, India, presented studies showing that the dityrosine bond cross-linking between two tyrosines is an oxidative marker and is present in aging and in many diseases. It is present in eye lens proteins α- and γB-crystallins formed through radical reactions and type I photosensitization.

Dr. Henry Forman from the University of Alabama, Birmingham, AL, reviewed the multiplicity of physiological responses modulated by oxidative signaling pathways. Some of the specific signaling components involved in such signaling and the effects of antioxidant enzymes were discussed. The principal points were "turn-off," components in signaling, such as antioxidant enzymes, spatial relationships, and deprotonation of cysteines to form a thiolate.

Dr. Sadis Matalon from the University of Alabama, Birmingham, AL, presented studies showing that modulation of surfactant protein-A (SP-A) either stimulates or inhibits NO production, depending on the activation state of the macrophage. SP-A stimulates alveolar macrophages to phagocytose and kill pathogens and is important in host defenses. SP-A may prove to be an effective therapy for both infectious and inflammatory diseases of the lung.

Dr. Chuanshu Huang from the New York University School of Medicine, Tuxedo, NY, presented a report on the role of UV-induced activation of extracellular signal-regulated kinases (ERKs), c-Jun NH_2-terminal kinases (JNKs), and p38 kinase phosphorylation of Akt and $p70^{S6k}$ involved in skin carcinogenesis. He showed that hydrogen peroxide and hydroxyl radical generation is involved in UV-induced phosphorylation of Akt and $p70^{S6k}$ and the activation of JNKs and p38 kinase, but not ERKs.

Dr. Debra Laskin from Rutgers University and University of Medicine & Dentistry, Piscataway, NJ, discussed the potential mechanisms regulating alveolar macrophage activity following ozone inhalation and the role of inflammatory mediators in toxicity. NO intermediates were shown to be a major effector molecule in ozone-induced injury.

Dr. Viswanathan Natarajan from Johns Hopkins School of Medicine, Baltimore, MD, discussed the critical role of enzyme phospholipase D (PLD) in intracellular signal transduction. ROS were shown to be involved in the activation of the PLD1 and PLD2 isoforms. In pulmonary epithelial and vascular endothelial cells, membrane phospholipids such as phosphatidic acid and its metabolic products were shown to play a central role in modulating endothelial and epithelial cell functions.

Dr. Naranjan Dhalla from the University of Manitoba, Winnipeg, Manitoba, Canada, reported that ischemia/reperfusion injury of the heart is associated with sarcoplasmic reticulum (SR) Ca^{2+} release, gene expression for SR proteins, and phospholamban. Alterations in cardiac contractile performance, SR function, and gene expression were attenuated by superoxide dismutase and catalase in reperfusate.

Dr. Brooke Mossman from the University of Vermont, Burlington, VT, reviewed the cell signaling pathways initiated by environmental particulates either indirectly through RONS or by the direct action of particulates with the cells. Cell signaling cascades leading to activation of the redox-sensitive transcription factors, nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), were discussed. The activation of these transcription factors results in increases in gene expression controlling cell injury or apoptosis, proliferation and/or cell survival, and inflammatory cytokines. These studies provided a causal relationship between these pathways and changes in epithelial cell phenotype linked to disease initiation.

Dr. Balaraman Kalyanaraman from the Medical College of Wisconsin, Milwaukee, WI, discussed the role of endothelial nitric oxide synthase (eNOS) in doxorubicin (DOX)-induced cardiomyopathy in cancer patients. DOX-induced apoptosis and eNOS transcription were inhibited by redox–metal chelators. These studies suggest the role of RONS in DOX-induced cardiotoxicity.

Dr. Valerian Kagan from the University of Pittsburgh, Pittsburgh, PA, emphasized the preferential oxidation of phosphatidylserine (PS) as an early event in apoptotic signaling. Depletion of Bcl-2 was associated selective oxidation of PS. Externalization of PS was shown to facilitate the labeling of apoptotic cells for recognition by macrophages and clearance.

Dr. John Eaton from the University of Louisville, Louisville, KY, presented studies showing that glycated proteins bind transition metals, such as copper and iron, and that such "glycochelates" accumulate within the vasculature in diabetes and catalytically inactivate endothelium-derived relaxing factor.

Dr. Paul Borm from the Institute for Environmental Health, Dusseldorf, Germany, presented a report on the mechanisms of neutrophil-induced DNA damage in respiratory epithelium. These studies demonstrate that neutrophils have the capacity to induce DNA damage in lung epithelial cells; however, in human nasal epithelium, no DNA damage was evident.

Dr. David Kamp from Northwestern University Medical School, Chicago, IL, reported on the role of mitochondrial dysfunction in asbestos-induced apoptosis. Asbestos-derived ROS were suggested to be involved in the mitochondrial death pathway, resulting in asbestos toxicity.

Dr. Vince Castranova from the National Institute for Occupational Safety and Health, Morgantown, WV, described mechanisms involved in the initiation and progression of silicosis in a nonoverload animal model. Lung damage, inflammation, NF-κB activation, cytokine production, alveolar type II epithelial cell activation, and fibrosis were monitored at different time periods. These responses were shown to be temporally associated with NO production by alveolar macrophages and type II epithelial cells. There was an anatomical association between areas of high NO production and the development of lung damage, inflammation, and fibrosis.

Dr. Nancy Colburn from the National Cancer Institute, Frederick, MD, presented a report on the role of AP-1, NF-κB, and RONS in skin carcinogenesis. Coordinated effects on tumor necrosis factor-α (TNF-α), NF-κB, and AP-1 pathways by RONS and abrogation of these effects by antioxidants were discussed.

Dr. Robert Floyd from the University of Health Science Center, Oklahoma City, OK, reported on studies in choline deficiency-induced hepatocarcinogenesis, and that α-phenyl-*tert*-butyl nitrone and some of its hydroxylated derivatives (the 4- and 3-hydroxylated compounds) prevent hepatocarcinogenesis in an animal model. The effect of nitrone compounds appears to be due to their ability to shift the apoptosis/neoplastic tendency balance toward apoptosis of the cells within preneoplastic lesions.

Dr. Bruce Pitt from the University of Pittsburgh, Pittsburgh, PA, presented evidence showing that NO signaling is associated with an increase in labile zinc, which in turn reduces the sensitivity of pulmonary endothelium to lipopolysaccharide-induced apoptosis. The antiapoptotic effects of NO were significantly inhibited by Zn^{2+} chelation with low doses of TPEN.

Dr. Berran Yucesoy from Ankara University, Ankara, Turkey, discussed the genetic polymorphisms of TNF-α and interleukin-1 (IL-1) and the role of regulating the production of these proinflammatory cytokines in silicosis severity. Polymorphisms of these genes showed independent and interrelated effects on the severity of silicosis.

Dr. Peter Ward from the University of Michigan, Ann Arbor, MI presented studies on the acute inflammatory response triggered by the deposition of IgG immune complexes. The inflammatory reaction caused severe tissue damage and activation of NF-κB with the generation of chemokines and cytokines. Endogenous IL-10, IL-13, and secreted leukocyte protease inhibitor (SLPI) were shown to be important regulators of the inflammatory response by reducing gene activation and the resultant generation of peptide mediators/cytokines and chemokines.

Dr. Matthew Grisham from Louisiana State University, Shreveport, LA reported on the role of NO in liver ischemia and reperfusion injury. He showed that eNOS-derived NO could modulate the expression of certain proinflammatory cytokines and limit tissue injury. Therapies involving NO donors or stimulants of eNOS expression were suggested useful in treating ischemia/reperfusion injury in the clinical setting.

Dr. Irfan Rahman from the University of Edinburgh, Scotland, U.K. reported that oxidants and TNF-α might activate NF-κB and AP-1 transcription factors, and enhance the expression of both proinflammatory and protective antioxidant genes. Remodeling of chromatin by acetylation/deacetylation of historic residues on the historic core of DNA was shown to be important in DNA binding and gene transcription. In human alveolar epithelial cells, oxidative stress and TNF-α altered historic acetylation/deacetylation, leading to the activation of NF-κB and AP-1 and the release of the proinflammatory cytokine IL-8. These TNF-α effects were associated with decreased GSH levels.

Dr. Augustine Choi from the University of Pittsburgh, Pittsburgh, PA presented evidence showing that carbon monoxide functions as a second messenger in biological systems, and that inducible isoforms of heme oxygenase-1 produced during the oxidative catabolism of heme confer protection *in vitro* and *in vivo* against oxidative cellular stress. Carbon monoxide was also shown to exert novel antiinflammatory and antiapoptotic effects dependent on the modulation of the p38 mitogen-activated protein kinase-signaling pathway.

Dr. Max Costa from the New York University School of Medicine, Tuxedo, NY, reported on the general principles involved in oxidative stress and molecular responses to toxic metals with special emphasis on the role of oxidative stress in nickel-induced genetic damage and mutations. The cross-linking of amino acids to DNA by nickel and the selective ability of nickel to silence the expression of genes located near heterochromatin were discussed. The effect of vitamin E on genotoxicity and mutations induced by nickel compounds and chromate was also discussed.

Dr. Petia Simeonova from the National Institute for Occupational Safety and Health, Morgantown, WV, reported on the carcinogenicity of arsenic and the modulation of signaling pathways initiating cell proliferation. c-Src was shown to be an important molecule involved in the initiation and the expression of genes related to cell-cycle regulation.

Dr. Xianglin Shi from the National Institute for Occupational Safety and Health, Morgantown, WV, reported on the important role of different chromium ions in carcinogenicity. Reduction of Cr(VI) by various enzymes, organelles, intact cells, and whole animals led to free radical production, DNA damage, activation of NF-κB, AP-1, p53, NFAT (nuclear factor of activated T cells), and vascular endothelial growth factor, tyrosine phosphorylation, apoptosis, cell growth arrest, and gene expression.

Dr. Tom Hei from College of Physicians & Surgeons, Columbia University, New York, NY, presented a talk on the mutagenic/carcinogenic mechanism of arsenic. His studies show a direct link to the induction of ROS by arsenite and genotoxicity showing extranuclear mitochondrial involvement.

Dr. James Mitchell from the National Cancer Institute, Bethesda, MD, reported on the protection of oxidative damage by nitroxides at nontoxic concentrations in *in vitro* and *in vivo* studies. Nitroxides were shown to have several functions for the protection of oxidative damage, such as superoxide dismutase mimicking, oxidation of reduced metals, termination of free radical chain reactions, drug-derived radical detoxification, and detoxification of ferryl and cupryl ions. Potential use of nitroxides in clinical conditions was stressed.

Dr. Chandan K. Sen from the Ohio State University Medical Center, Columbus, OH, reviewed the potent neuroprotective properties of vitamin E. He highlighted that this information has been derived from the study of α-tocopherol. It was discussed that in nature, the vitamin E family consists of eight members broadly categorized as tocopherols and tocotrienols, and that tocotrienols are abundantly found in palm oil, cereal grains, and rice bran. Next, he presented studies from his laboratory to show that tocotrienol is a better and highly potent neuroprotective form of vitamin E that functions at nanomolar concentrations. Molecular mechanisms by which α-tocotrienol exerts its neuroprotective effects were discussed. It was also shown that tocotrienol is bioavailable to the brain by oral feeding.

Dr. A.K. Susheela from the Fluorosis Research Foundation, Delhi, India, reported on the reversal of fluoride-induced cell injury and disease by supplementation of the diet with essential nutrients and antioxidants. Treated patients, monitored at frequent intervals up to 1 year, were shown to have decreased physical and clinical manifestation of fluorosis.

Dr. Etsuo Niki from the National Institute of Advanced Industrial Science and Technology, Ikeda, Japan, discussed the effects of radical-scavenging antioxidants in ameliorating cardiovascular disease. He reviewed different mechanisms of low-density lipoprotein oxidation and efficacy of some antioxidants.

Dr. Harold Swartz from Dartmouth Medical School, Hanover, NH, presented useful technical information concerning *in vivo* electron paromagnetic resonance (EPR) spectroscopy with emphasis on the relationship to experiments that are useful for the investigations of RONS.

Dr. Jay Zweier from Johns Hopkins University School of Medicine, Baltimore, MD, presented the important role of noninvasive *in vivo* EPR measurement, spatial mapping, and imaging in understanding cellular injury and pathophysiology in mouse to man. Spatial EPR imaging enables three-dimensional mapping of the distribution of a given free radical, whereas spectral–spatial EPR imaging enables mapping of the spectral information at each spatial position. From the observed line width, the localized tissue oxygenation can be determined. By using spatial EPR imaging, the distribution and metabolism of nitroxide radicals within the major organs of the body of living mice were visualized and anatomically coregistered by proton MRI enabling *in vivo* mapping of the redox state and rate of radical clearance.

Dr. Stephen Leonard from the National Institute for Occupational Safety and Health, Morgantown, WV, presented correlative results using conventional bioassays with EPR spectroscopic and imaging studies on changes occurring in acute asbestosis. *In vivo* pharmacokinetics measurements of redox status will be valuable in the study and treatment of lung diseases.

Dr. Ke Jian Liu from the University of New Mexico, Albuquerque, NM, presented a study on the application of 4-hydroxybenzoic acid oxidation to 3,4-dihydroxybenzoic acid as a reliable indicator for the formation of hydroxyl radicals *in vivo* during cerebral ischemia and reperfusion.

Dr. Andrei Komaro from the George Washington University Medical Center, Washington, DC, discussed the *in vivo* detection of NO in liver, kidney, blood, and urine of mice exposed to endotoxin. Potential artifacts involved in dithiocarbamate–iron complexes as NO trapping agent were discussed.

The meeting was attended by 214 scientists and clinicians from academia, state and federal governments, and industries representing 14 different countries.

ABBREVIATIONS

AP-1, activator protein-1; DOX, doxorubicin; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; IL, interleukin; JNK, c-Jun NH_2-terminal kinase; NF-κB, nuclear factor-κB; NO, nitric oxide; PLD, phospholipase D; PS, phosphatidylserine; RONS, reactive oxygen/nitrogen species; ROS, reactive oxygen species; SP-A, surfactant protein-A; SR, sarcoplasmic reticulum; TNF-α, tumor necrosis factor-α.

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