focal lesions however were identified in all dose groups during a 6- month repeat dose study suggesting an early indicator of the hepatocellular carcinogenicity finding. Investigations are currently focused on identifying RO-450-specific changes in shorter term studies that might yield insight into the nonogenotoxic mechanism by which hepatocellular neoplasia develop.

291 REDOX REGULATION OF GAMMA-IRRADIATION INDUCED APOPTOSIS BY CYTOCHROME C–CARDIOLIPIN COMPLEX IN HELA CELLS.

N. A. Belikova[1, 2], J. Jiang[1, 2], Y. Y. Tyurina[1, 2], Q. Zhao[1, 2], M. W. Epperly[2, 3], J. Greenberger[2, 3] and V. E. Kagan[1, 2]. *[1]Center for Free Radical and Antioxidant Health and Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, [2]Center for Medical Countermeasures against Radiation, University of Pittsburgh, Pittsburgh, PA and [3]Department of Radiation Oncology, University of Pittsburgh, Pittsburgh, PA.*

In apoptosis, cytochrome c (cyt c) released from mitochondria into the cytosol, is central to apoptosome formation and caspase activation. Recently, we established that cyt c participates in apoptosis acting as a cardiolipin (CL)-specific peroxidase. The CL oxidation products thus formed are essential for the release of pro-apoptotic factors from mitochondria. While assembly of apoptosomes requires only minimal amounts of cyt c, catalysis of CL oxidation is strongly dependent on its content and availability for CL in mitochondria. Thus we hypothesized, the concentration of cyt c can be a determinant of cell's sensitivity to irradiation induced apoptosis. To test the hypothesis, we used siRNA approach to engineer HeLa cells with a lowered content of cyt c (14% of its amount in parental cells), HeLa 1.2 cells. γ-irradiation (in doses up to 40 Gy) induced apoptosis - as revealed by caspase-3/7 activation and phosphatidylserine externalization - was proportional to the cyt c content in HeLa 1.2 cells. Using a fluorescence HPLC-based Amplex Red assay and ESI-MS analysis, we found that irradiation caused selective accumulation of CL hydroperoxides. HeLa 1.2 cells responded by a lower irradiation-induced accumulation of CL oxidation products. No release of a pro-apoptotic factor Smac/DIABLO was detected in cyt c-deficient cells after irradiation, whereas Bax translocation was the same as in HeLa cells. Combined, our results demonstrate that cyt c is an important catalyst of CL peroxidation, critical to the execution of apoptotic program. This redox catalytic role of cyt c in irradiation induced apoptosis can be useful for the development of new radioprotectors and radiosensitizers. Supported by NIH NIAID U19 AI068021, NIH HL70775 and The Human Frontier Science Program.

292 PEROXIDASE COMPLEXES OF CYTOCHROME C WITH ANIONIC LIPIDS: STRUCTURAL PRE-REQUISITES, MECHANISMS, AND CYTOTOXIC EFFECTS.

I. I. Vlasova, A. A. Kapralov, J. Jiang, L. V. Basova, N. A. Belikova, V. A. Tyurin, Y. Y. Tyurina, J. Martin, A. Glumac, H. Bayir and V. E. Kagan. *Center for Free Radical and Antioxidant Health, EOH, University of Pittsburgh, Pittsburgh, PA.*

Interaction of cytochrome c (cyt c) with a mitochondria-specific cardiolipin (CL) confers peroxidase activity on the protein resulting in selective CL oxidation and release of proapoptotic factors. Because the complex cyt c/CL is stabilized by a combination of electrostatic and hydrophobic interactions, we determined the extent to which other anionic lipids - phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylinositolphosphates (PIP), and phosphatidylcholine (PC) as a control - are effective in inducing the peroxidase activity of cyt c. EPR spectroscopy of nitrosylated cyt c, optical spectroscopy and measurements of tryptophan fluorescence demonstrated that cyt c interaction with anionic lipids induced protein unfolding accompanied by an exchange and loss of axial ligands of heme iron so that cyt c heme became more accessible for the interaction with small molecules like NO or H2O2. Using several peroxidase substrates we showed that all anionic lipids activated peroxidase activity of cyt c in a dose-dependent manner with the efficiency decreasing in the row: CL=PA>PIP2>PIP3>PS. Recombination of protein-derived radicals formed in peroxidase reaction caused oligomerization of cyt c and formation of protein-lipid aggregates detectable by PAGE and Western blotting. In line with this, Western blotting revealed the formation of cyt c aggregates after its incubation in the presence of H2O2 with membrane (but not cytosolic S-100) fraction of brain homogenates. Oxidation products of anionic lipids were detected after induction of apoptosis in HeLa cells and mouse embryonic wild type cells but not in cyt c-deficient cells. Overall, our study identified anionic lipids as physiologically relevant regulators of peroxidase activity of cyt c in mitochondria and other cell compartments, particularly during apoptosis. Supported by NIH U19 AI068021, HL 070807, NIOSH OH008282, AHA0535365N, PA Dept. of Health SAP 4100027294.

293 FREE FATTY ACIDS FORM PEROXIDASE COMPLEXES WITH CYT C: ROLE IN MITOCHONDRIAL OXIDATIVE STRESS AND DAMAGE.

A. Kapralov, I. Vlasova, N. Belikova, W. Feng, L. Basova, J. Martin, A. Glumac, H. Bayir and V. Kagan. *Center for Free Radical & Antioxidant Health, EOH, University of Pittsburgh, Pittsburgh, PA.*

Recently, we reported that negatively charged phospholipids- cardiolipin,phosphatidylserine-form strong complexes with cyt c in which the hemoprotein acts as a potent peroxidase capable of selective oxidation of respective phospholipids (Kagan et al., Nature Chem. Biol, 2005). Both electrostatic and hydrophobic interactions were found essential for the complexes formation. Free fatty acids (FFAs) carry both a negatively charged carboxy-group and long chain hydrophobic moiety. Because FFA accumulation occurs in membranes and biofluids in many dyslipidemias and other disease conditions, we hypothesized that they can stimulate peroxidase activity of cyt c. Here we report, that oleic acid effectively stimulated peroxidase activity of cyt c as evidenced by oxidation of three typical peroxidase substrates: Amplex Red, etoposide, and luminol. The peroxidase activation of cyt c occurred only at ratios of oleic acid/cyt exceeding 50:1. Generation of characteristic protein–derived radicals was confirmed by EPR spectroscopy of cyt c/oleic acid incubated with H2O2. Oligomerization of cyt c/FFA complexes was demonstrated by Western blotting. By utilization of immuno-spin trapping technique, we found the production of protein-immobilized DMPO nitrone adducts in the PAGE bands corresponding to oligomeric forms of cyt c. Two lines of evidence suggest interaction of cyt c with oleic acid unfolds the protein resulting in a greater accessibility of its heme: 1) in low temperature EPR spectra of heme-nitrosylated oleic acid/cyt c complexes, a signal of penta-coordinate heme-iron was detectable, and 2) increased fluorescence of tryptophan quenched in native enzyme by the heme moiety. Increased levels of cyt c-associated peroxidase activity were found in isolated mitochondria and in cells incubated with oleic acid suggesting that the complexes may function as a peroxidase in vivo. Supported by grants from NIH U19 AI068021, NIOSH OH008282, AHA0535365N, Pennsylvania Department of Health SAP 4100027294.

294 OXIDIZED CARDIOLIPIN AS A NEW BIOMARKER OF GAMMA-IRRADIATION-INDUCED APOPTOSIS.

Y. Y. Tyurina[1], V. A. Tyurin[1], M. W. Epperly[2], J. S. Greenberger[2] and V. E. Kagan[1]. *[1]Center for Free Radical and Antioxidant Health and Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA and [2]Department of Radiation Oncology, University of Pittsburgh, Pittsburgh, PA.*

Recently, we demonstrated that cytochrome c acts as a cardiolipin (CL) - specific peroxidase in mitochondria early in apoptosis and that CL oxidation products are required for the release of proapoptotic factors. Accordingly, gamma-irradiation initiates apoptotic response in HeLa cells and CL oxidation is critical to the execution of apoptotic program in these cells. We hypothesized that accumulation of oxidized CL can potentially be used in vivo as an early biomarker of apoptosis initiated by total body irradiation (TBI). As intestines are one of sensitive targets of irradiation, we studied phospholipid oxidation in guts isolated from control and irradiated mice. To this end C57BL/6NHsd female mice were subjected to 10 and 15 Gy of TBI and sacrificed 24 h thereafter. We found that TBI caused a dose-dependent apoptosis as revealed by caspase-3/7 activation. Moreover, a significant dose-dependent decrease in sphingomyelin content was detected in guts of TBI exposed mice thus confirming activation of sphingomyelinase typically involved in ceramide-dependent apoptotic pathway. TBI induced apoptosis was accompanied by oxidative stress as evidenced by depletion of two major intracellular antioxidants - ascorbate and GSH. Using our new protocol, oxidative lipidomics, we characterized and quantitatively assessed the formation of hydroperoxides of different classes of phospholipids and found that only CL, a mitochondria specific phospholipids (but not other phospholipids) undergo significant oxidation in mouse guts after TBI. Thus, early accumulation of CL hydroperoxides can be used in vivo as a biomarker of TBI induced apoptosis, useful for the development of new radioprotectors and radiosensitizers. Supported by NIH NIAID U19 AI068021, NIH HL70755.

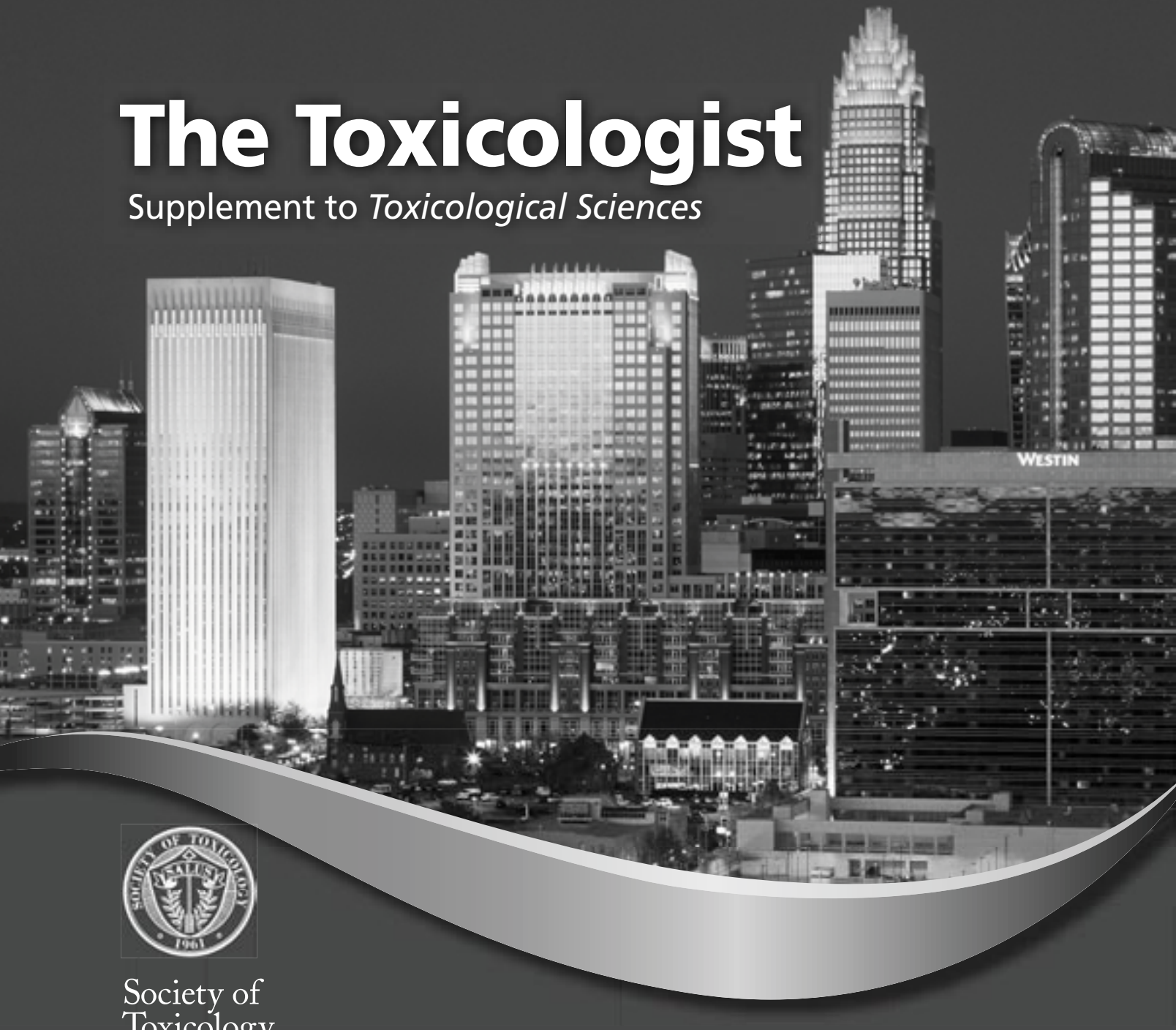
295 UNMASKING PEROXIDASE ACTIVITY OF CYTOCHROME C IN MITOCHONDRIA: ROLE OF CARDIOLIPIN.

L. V. Basova[1], I. V. Kurnikov[1], L. Wang[2], V. B. Ritov[2], I. I. Vlasova[1], N. A. Belikova[1], A. A. Pacheco[3], H. Bayir[1], D. H. Waldeck[2] and V. E. Kagan[1]. *[1]EOH, University of Pittsburgh, Pittsburgh, PA, [2]Chemistry, University of Pittsburgh, Pittsburgh, PA and [3]Chemistry, University of Wisconsin, Milwaukee, WI.*

Redox properties of cyt c are critical to its normal electron transport function in mitochondria, as well as to its participation in apoptotic signaling via the peroxidase oxidation of two anionic phospholipids, cardiolipin (CL) and phosphatidylserine

The Toxicologist

Supplement to *Toxicological Sciences*



Society of Toxicology

46th Annual Meeting and ToxExpo™

Charlotte, North Carolina

An Official Journal of the Society of Toxicology

www.toxsci.oxfordjournals.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 96, Number 1, March 2007

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 46th Annual Meeting of the Society of Toxicology, held at the Charlotte Convention Center, Charlotte, March 25–29, 2007.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 449.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 480.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190

www.toxicology.org