the induction of apoptosis. To investigate NO-mediated cellular damage in target cells, we co-cultured activated RAW 264.7 macrophages with a Chinese hamster ovary cell line (AS52). AS52 cells have been reported to be sensitive to reactive oxygen intermediates. Cells were grown in Ham's F12 media concurrently at varying ratios of AS52 to RAW 264.7 cells. Macrophages were activated with lipopolysaccharide (LPS). In order to clarify events induced in AS52 cells various parameters were examined, including p53 accumulation, bcl-2 levels, nitrotryosine levels, and cell death by necrosis and by apoptosis.

733 NITRIC OXIDE BUT NOT NITROSOTHIOLS INHIBIT TERT-BUTYL HYDROPEROXIDE/ HEMOGLOBIN-INDUCED OXIDATIVE STRESS AND CYTOTOXICITY.

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Nitric oxide (NO) can interact with a number of biomolecular carriers to form products that retain biological activity. It has been suggested that physiological effects of nitric oxide (e.g., those associated with endothelium-derived relaxing factors) may be related to NO-adducts, such as a nitrosothiols [Creager MA. Roddy MA. Boles K. Stamler JS. N-acetylcysteine does not influence the activity of endothelium-derived relaxing factor in vivo. Hypertension. 29(2):668-72, 1997]. Antioxidant effects of NO are believed to play an important role in its multifunctional physiological activity. The two major antioxidant mechanisms of NO are: (i) direct radical scavenging, and (ii) interaction with hemoproteins that prevent formation of potent oxidants, oxoferryl-associated radicals. In this study, we compared effects of an NO-donor, NOC-15, and two nitrosothiols, NO-GS and NO-Cys, on cytotoxicity and oxidative stress induced by tert-butyl hydroperoxide (tBuOOH)/oxyhemoglobin (oxyHb) in rat mesenteric smooth muscle cells. We found that tBuOOH/oxyHb induced pronounced peroxidation of major membrane phospholipids -phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine in the cells as measured by a metabolically integrated oxidation-sensitive fluorescent probe, cis-parinaric acid (PnA). PnA-labeled phospholipids in cells were significantly protected against oxidation by NOC-15. Neither NO-Cys nor NO-GS were able to inhibit tBuOOH/oxyHb-induced oxidation. Similarly, the tBuOOH/oxyHb-induced decrease in cell survival was not affected by either NO-GS or NO-Cys. A complete protection was, however, provided by NOC-15. Our ESR and spectrophotometric measurements demonstrated formation of heme-nitrosylated Hb in the presence of NOC-15 and lack of heme nitrosylation by either NO-GS or NO-Cys. We conclude that nitrosothiols do not act as antioxidants against hemoglobin-catalyzed oxidative stress induced by peroxides.

734 EARLY INCREASE IN THE HEPATOCELLULAR LABILE IRON POOL PRECEDES NITROFURANTOIN-INDUCED OXIDATIVE CELL INJURY.

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The labile iron pool (LIP) represents the non-ferritin bound, redox-active iron that has been implicated in catalyzing the formation of •OH radicals during oxidative stress. Here we examined whether the LIP can be monitored in primary cultures of murine hepatocytes and whether alterations in LIP iron are related to the oxidative damage inflicted by the redox cycling drug nitrofurantoin (NFT). Early changes in the LIP were monitored with the metal-sensitive fluorescent probe calcein (CA), which is retained in hepatocytes and the fluorescence of which is quenched upon binding to Fe^{2+}. Immediately after the addition of NFT (300 μM), intracellular $O_2^{\cdot-}$ production was massively increased. Simultaneously, the CA fluorescence signal was reduced by 30%, indicating that the amount of LIP-associated ferrous iron had increased. Prolonged exposure (>60 min) to NFT caused protein oxidation and resulted in lethal cell injury. Furthermore, addition of the cell-permeable ferrous iron chelator, 2,2′-bipyridyl (100 μM) not only prevented the quenching of CA fluorescence but also protected from NFT toxicity. These results indicate that the NFT-induced increase in LIP-associated iron is an essential event that precedes oxidative cell damage and that can be monitored in hepatocytes. LIP-associated free iron may become an important target for antioxidant treatment against chemically-induced oxidative cell injury.

735 THE CYTOPROTECTIVE MECHANISMS OF DIHYDROXYACETONE TOWARDS HEPATOCYTE INJURY BY A WIDE RANGE OF XENOBIOTCS.

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Previously we showed that dihydroxyacetone (DHA) was more effective than other antidotes at preventing cyanide toxicity in vivo or hepatocytes in vitro (Toxicol.Appl.Pharmacol.138,186–91 (1996)). Hepatocyte respiration was rapidly restored before ATP levels recovered, probably because cyanide was trapped by DHA to form a cyanohydrin. However, hepatocyte injury induced by hypoxia or respiratory inhibitors, eg. antimycin, was also prevented and ATP levels partly recovered without affecting respiration (Chemico.Biol.Interac.98,27–44 (1995); Biochem.Biophys.Acta. 1269,153–61 (1995)). Reductive stress and cytotoxic oxygen activation were more critical for cell injury than ATP depletion and DHA fully restored hepatocyte redox potential (lactate:pyruvate ratio). A hepatic triokinase metabolized DHA to the glycolytic intermediate, DHA phosphate which oxidized NADH to form glycerol phosphate via glycerol phosphate dehydrogenase. We have now shown that DHA prevents H_2S cytotoxicity probably because H_2S was trapped by DHA. DHA also protected hepatocytes from endogenous H_2O_2 generated by nitrofurantoin or tyramine as well as tyramine induced lipid peroxidation. DHA also prevented hepatocyte cytotoxicity induced by various metals including cadmium or mercuric chloride. The formation of reactive oxygen species as determined by luminol chemiluminescence was also prevented suggesting that DHA scavenges reactive oxygen species. In conclusion the cytoprotective properties of DHA towards a wide range of xenobiotics can be attributed to its ability to maintain cellular redox potential and energy status as well as metal complexing and antioxidant properties.

736 EFFICACY OF TAURINE AND HYPOTAURINE AS HYDROXYL RADICAL SCAVENGERS.

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We previously reported that taurine (2-aminoethanesulfonic acid) levels increase in alveolar macrophages (AM) under oxidant stress, presumably by mobilizing taurine from bound intracellular stores (Tox. Appl. Pharm. 105:55–65, 1990). In another study, AM loaded with taurine prior to oxidant stress were found to be less susceptible to oxidant injury (J. Nutr. Biochem. 2: 308–313, 1991). In this study, we investigated the radical scavenger activity of taurine, and its metabolic precursor hypotaurine (2-aminoethanesulfinic acid), both of which may act as radical scavengers in the cell. Electron spin resonance (ESR) was used to investigate the reaction of taurine and hypotaurine with hydroxyl radicals (·OH). The Fenton-reaction ($Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\cdot}OH + {}^{-}OH$) and the Cr(V)-mediated Fenton-like reaction ($Cr(V) + H_2O_2 \rightarrow Cr(VI) + {}^{\cdot}OH + {}^{-}OH$) were used as sources of ·OH radicals. To further explore the ·OH radical scavenger activity of taurine and hypotaurine, their ability to prevent lipid peroxidation was evaluated using linoleic acid as a model lipid and silica as an occupational source of ·OH radicals and other oxidants. The results show that taurine does not scavenge ·OH radicals nor prevent lipid peroxidation at concentrations up to 20 mM. In contrast, hypotaurine scavenged ·OH radicals with reaction rate constant of $k = 1.6 \times 10^{10} M^{-1}s^{-1}$ and protected against lipid peroxidation in a concentration-dependent manner from 0.5–20 mM. The rate constant for hypotaurine is comparable with other efficient cellular ·OH radical scavengers, such as ascorbate and glutathione. These results suggest that hypotaurine, not taurine, was responsible for the decrease in lipid peroxidation in oxidant-stressed AM whereas taurine may have contributed to membrane integrity via its interaction with membrane lipids. Further experiments are needed to explore the possible role of hypotaurine as a cellular antioxidant and whether intracellular hypotaurine oxidation accounts for the increase in intracellular taurine levels previously observed in oxidant-stressed AM.

737 ANTIOXIDANT AND CYTOPROTECTIVE PROPERTIES OF D -TAGATOSE AGAINST NITROFURANTOIN TOXICITY TO CULTURED MURINE HEPATOCYTES.

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D-Tagatose is a zero-energy producing ketohexose that is a powerful cytoprotective agent against prooxidant-induced cell injury. To further explore the underlying mechanisms of cytoprotection, we investigated the effects of D-tagatose on both the generation of $O_2^{\cdot-}$ and the consequences of oxidative

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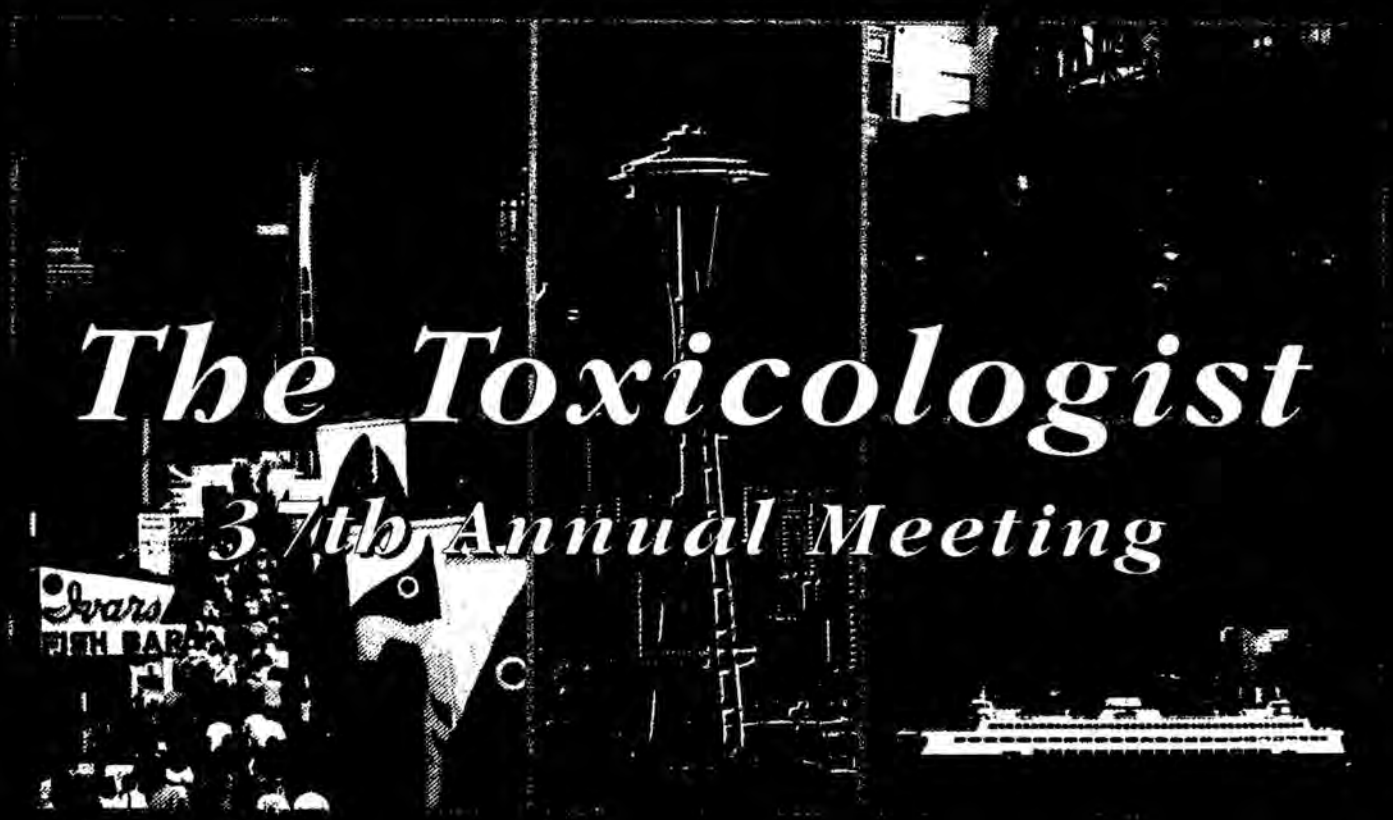

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshop, roundtable, and poster sessions of the 37th Annual Meeting of the Society of Toxicology, held at the Washington State Convention Center, Seattle, Washington, March 1-5, 1998.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 407.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 433.

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