201 SUPEROXIDE-INDUCED REDUCTION OF COENZYME Q AND ITS INTERACTION WITH VITAMIN E SYNERGISTICALLY PROTECTS HUMAN KERATINOCYTES AGAINST OXIDATIVE STRESS.

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Enhanced production of superoxide is commonly associated with the induction of oxidative stress. It has been sugested that, in the presence of ubiquinone, superoxide can act as an antioxidant *via* reduction of ubiquinone to semiubiquinone/ubiquinol. The latter couple can scavenge radicals directly or act as an antioxidant through its interactions with vitamin E phenoxyl radical, which leads to vitamin E recycling. To investigate the hypothesis, we used normal human epidermal keratinocytes (NHEK) supplemented with vitamin E, ubiquinone or combination thereof. NHEK were exposed to oxidative stress induced by lipid-soluble azo-initiator of peroxyl radicals, AMVN, in the presence and in the absence of superoxide-generating system (xanthine oxidase/xanthine). We established conditions under which either vitamin E alone or ubiquinone alone has only caused a slight protection against AMVN-induced oxidation of NHEK phospholipids as assayed by metabolically-integrated oxidation-sensitive fluorescent parinaric acid (PnA). The protection of NHEK supplemented with vitamin E was not enhanced by ubiquinone; however in the presence of xanthine oxidase/xanthine, synergistic protection against AMVN-induced phospholipid peroxidation was observed as compared to the effects of either vitamin E alone or ubiquinone alone. Xanthine/oxidase induced production of superoxide was able to reduce ubiquinone hence contribute to effective vitamin E recycling. We conclude that the mechanism of superoxide-driven reduction of ubiquinone to semiubiquinone/ubiquinol can facilitate recycling of vitamin E, by which it enhances antioxidant protection of keratinocytes.

202 PHENOL INDUCED *IN VIVO* OXIDATIVE STRESS IN SKIN: EVIDENCE FOR ENHANCED FREE RADICAL GENERATION, THIOL OXIDATION AND ANTIOXIDANT DEPLETION.

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A variety of phenolic compounds are utilized in industry (*e.g.*, for production of phenol-formaldehyde resins, paints and lacquers, cosmetics and pharmaceuticals). They can be toxic to skin (*i.e.*, can cause rash, dermal inflammation, contact dermatitis, leucoderma, and/or cancer promotion). The biochemical mechanisms for dermal toxicity of phenolic compounds are not well understood. We hypothesized that enzymatic one-electron oxidation of phenol compounds, resulting in generation of phenoxyl radicals, may be an important contributor to dermal toxicity. To test this hypothesis, we monitored *in vivo* the formation of α-phenyl-N-*tert*-butylnitrone (PBN)-spin-trapped radical adducts by ESR spectroscopy, measured glutathione (GSH), protein thiols, vitamin E and total antioxidant reserves in skin of B6C3F1 mice topically treated with phenol and compared the responses with those produced by phenol in mice with diminished levels of GSH. We found that dermal exposure to phenol (3.5 mmol/kg, 100 µl on the shaved back, for 30 min) caused oxidation of GSH and protein thiols and decreased vitamin E and total antioxidant reserves in skin. The magnitude of the phenol-induced generation of PBN-spin-trapped radical adducts in skin of mice with diminished levels of GSH (pre-treated with 1,3-bis(2-chloroethyl)-1-nitrosourea, BCNU, an inhibitor of glutathione reductase) was markedly higher compared to those treated with phenol alone. BCNU pre-treated mice also exhibited increased inflammatory cell infiltrates in skin after topical exposure to phenol. Since dermal exposure to phenol produced ESR-detectable PBN spin-trapped signals of lipid-derived radicals, we conclude that redox-cycling of a one-electron oxidation product of phenol, its phenoxyl radical, is involved in oxidative stress and dermal toxicity *in vivo*.

203 REGULABLE REDUCTION OF ENDOGENOUS OXIDATIVE DNA DAMAGE IN TRANSGENIC MICE CARRYING THE DNA REPAIR GENE FORMAMIDOPYRIMIDINE DNA GLYCOSYLASE (FPG).

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8-Oxoguanine (8oxoG) is a pervasive oxidative DNA lesion formed by endogenous oxidative stress and enhanced by drugs and environmental chemicals that initiate the formation of reactive oxygen species. We engineered transgenic mice to have regulable expression of the bacterial DNA repair gene formamidopyrimidine DNA glycosylase (fpg) by using the tetracycline-regulable gene expression system. We generated mice in which the tetracycline-regulable transcriptional activator (tTA) was placed under the control of the human beta-actin promoter, and mice in which fpg was placed under the control of the tetO promoter. In this first report of mice with increased in vivo repair of an oxidative DNA lesion, we demonstrate that neonatal brains from compound transgenic actin-tTA::TetO-fpg have an 8oxoG level which is half that in wild-type controls ($p < 0.001$). The extent of extra repair could be regulated by in vivo administration of the tetracycline analogue doxycycline during gestation and the early postnatal period. The amount of 8-oxoG in doxycycline-treated compound transgenic actin-tTA::TetO-fpg mice remained elevated at a level similar to that in singly transgenic actin-tTA transgenic controls, and was significantly higher than that in the compound transgenic actin-tTA::TetO-fpg mice which did not receive doxycycline ($p = 0.001$). Since the accumulation of 8-oxoG has been associated with neurodegenerative disease, cancer and birth defects, these animals provide a novel model to test the mechanistic role of the 8-oxoG lesion in such conditions (Support: Canadian Institutes for Health Research, National Ataxia Foundation, University of Rome.)

204 4-HYDROXYNONENAL INHIBITS INTERLEUKIN-6 PRODUCTION IN RAT KUPFFER CELLS.

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4-Hydroxynonenal (4-HNE) is an aldehydic product of lipid peroxidation implicated in the pathogenesis of prooxidant-induced liver damage. This aldehyde affects many diverse biological systems resulting in disregulation of normal cell function. During liver damage, Kupffer cells are activated and release various mediators that are involved in protection and regeneration of the liver. In this context, interleukin-6 (IL-6) has been shown to initiate hepatocyte proliferation and inhibit the fibrotic response upon chemical insult. This study examined the effects of 4-HNE on rat Kupffer cells and demonstrated that 4-HNE is a potent inhibitor of IL-6 production. Experiments utilized Kupffer cells preincubated with various concentrations of 4-HNE (0-10 µM) for 1 hour and subsequently activated with 500 ng/ml of bacterial lipopolysaccharide (LPS). Other experiments used Kupffer cells first activated with LPS (500 ng/ml) for 1 hour followed by exposure to varying doses of 4-HNE (0-10 µM). Using an enzyme-linked immunosorbent assay (ELISA), IL-6 cytokine production was measured at the 4 and 8 hour time points in both treatment groups. 4-HNE inhibited IL-6 production in a concentration-dependent manner in both groups. A 50% reduction in IL-6 protein production at a dose of 1 µM 4-HNE was demonstrated in both treatment groups at 8 hours. IL-6 mRNA levels were also analyzed by the sensitive Quantigene assay in both groups at the 8 hour time point. RNA levels were also decreased in a concentration-dependent manner in both treatment groups, thereby, demonstrating that 4-HNE inhibition of IL-6 production occurs at the transcriptional level. The mechanism of 4-HNE inhibition of IL-6 protein and RNA is presently being determined through gel-shift analysis of NF-kB activation studies. Decreased production of IL-6 by 4-HNE may compromise liver regeneration during periods of chronic lipid peroxidation. (Supported by AA05536 to SWL and AA09300 to DRP.)

205 GLUTATHIONE AND ASCORBATE IN THE OLFACTORY EPITHELIUM OF ALACHLOR-TREATED RATS.

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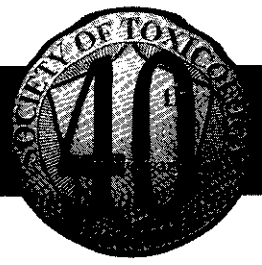
Alachlor (ALA), a chloracetanilide herbicide known to cause tumor formation in the olfactory epithelium (OE) of Long Evans rats, has previously been shown to be metabolized to a quinone imine metabolite (QI) in rat OE. QI are known to bind glutathione and could thereby produce an oxidative stress response. We examined this possibility with the hypothesis that oral administration of ALA induces oxidative stress in OE of rats. To test this hypothesis, we measured reduced glutathione (GSH), oxidized glutathione, and ascorbate levels in the OE of rats administered ALA in the diet (126 mg/kg/day) for 1, 2, 4, and 30 days. GSH levels showed an initial 35% decrease after 1 day of treatment. After two or four days of dietary exposure, GSH levels in the OE of treated animals were about 50% higher than the control levels. After 30 days of treatment, GSH levels were no longer significantly different between treated and control groups. The basal level of ascorbate in control OE was approximately 6 times higher than the ascorbate level in control liver. Ascorbate levels in OE paralleled changes in GSH levels. Ascorbate decreased by 35% in animals treated for 1 day. Animals treated for 2 and 4 days displayed increases in ascorbate of about 50%. At a lower dose of alachlor (63 mg/kg, 1 day), GSH and ascorbate levels were reduced by 18% and 30%, respectively. Preliminary



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Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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