618 **REACTION OF OZONE WITH LIPOSOMES CONTAINING UNSATURATED PHOSPHOLIPIDS.** J Santrock, R A Gorski, and J F O'Gara. Biomedical Science and Analytical Chemistry Departments, General Motors Research Laboratories, Warren, Michigan. Sponsor: M J Olson.

While considerable effort has been expended on determining the health effects of exposure to ozone (O_3), little is known about the chemical events responsible for toxicity. We studied the reaction of ozone with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in liposomes. Reaction of O_3 with the carbon-carbon double bond in POPC yielded an aldehyde and a hydroxyhydroperoxide (-CH(OH)(OOH)). The hydroxyhydroperoxide eliminated H_2O_2 to give a second aldehyde. Thus, two aldehydes were produced: nonanal and 1-palmitoyl-2-(9'-oxononanoyl)-sn-glycero-3-phosphocholine (PN1PC). Upon further ozonolysis, the aldehydes were oxidized to the corresponding carboxylic acids. The mechanisms of these reactions were deduced from a material balance and from the pattern of labeling in the products when $^{18}O_3$ was used as a reactant. An ozone-sensitive reactant in the intervesicular space was unaffected (1) as long as sufficient unsaturated fatty acid remained in the membrane and (2) the liposome remained intact. Accumulation of ozonolysis products in the membrane above a threshold concentration (~ 10 mol %) resulted in lysis of the liposomes; PN_1PC caused lysis.

619 REACTIONS OF OZONE WITH VITAMIN E. D C Liebler, S Matsumoto, and M Matsuo. Dept. Pharmacol. & Toxicol., Univ. of Arizona, Tucson, AZ and Tokyo Metropolitan Inst. Gerontology, Tokyo, Japan.

The biological antioxidant α-tocopherol (αTH; vitamin E) is an important protectant against oxidative lung injury by inhaled ozone. To investigate the reactions of ozone with αTH in a chemically defined model system, we bubbled ozone through acetonitrile or acetonitrile/aqueous solutions of αTH at ambient temperature. Products were analyzed and purified by reverse phase HPLC. Ozone oxidized αTH to α-tocopherolquinone in low (< 5%) yield, apparently via the intermediate 8a-hydroxytocopherone. Similarly small amounts of epoxy-8a-hydroperoxytocopherones and their hydrolysis products epoxy-tocopherolquinones also were detected. Two principal reaction products, which have been characterized by UV-vis and infrared spectroscopy, mass spectrometry, and ^{1}H- and ^{13}C-NMR spectroscopy were tentatively identified as products of oxidative cleavage of the αTH phenol ring. These products are not known to be formed from αTH by other oxidants and are produced in relatively high (≈40%) yield. The ring-opened products are not formed from tocopherolquinone, as oxidation of the quinone yielded different products. Unique products of ozone-αTH reactions may provide useful biochemical markers of ozone exposure to tissues. Supported in part by the Tokyo Metropolitan Government.

620 EFFECTS OF VITAMIN E AND LINOLEIC ACID SUPPLEMENTATION ON LIPID PEROXIDATION AND CYTOTOXICITY IN HUMAN KERATINOCYTES. H Wey and M Woolery. Cellular Toxicology, ETB, DBBS, NIOSH, CDC, Cincinnati, OH. SPONSER: M Toraason.

Cultured human keratinocytes (HK) grown in serum-free medium become deficient in polyunsaturated fatty acids (PUFA) derived from linoleic acid (LA) and this renders HK less responsive to lipid peroxidation and cytotoxicity induced by t-butyl hydroperoxide (BHP). We have previously shown that linoleic acid supplementation (LA-SUP) reverses this condition. Since the growth medium also lacks the antioxidant vitamin E, we investigated the effects of vitamin E succinate supplementation (E-SUP) on BHP-induced lipid peroxidation and cytotoxicity in HK. HK were cultured in medium (modified MCDB153) with combinations of E-SUP (2uM) and/or LA-SUP (10uM) for 5 days prior to a 2 hour exposure to BHP (1mM). Lipid peroxidation was quantitated by the appearance of thiobarbituric acid-reactive substances (TBARS) and cytotoxicity by the appearance of lactate dehydrogenase (LDH) in the incubation buffer. BHP treatment of unsupplemented HK did not affect LDH release, but significantly increased TBARS. BHP treatment of LA-SUP cells resulted in a further increase in TBARS release (3-fold), and also increased LDH release (2-fold) indicating a cytotoxic effect. E-SUP prevented the effect of BHP on LDH release in LA-SUP cultures. E-SUP also attenuated the effect BHP on TBARS release, but did not prevent the ability of LA-SUP to increase TBARS release in response to BHP. These results suggest that LA and E nutrition of HK should be considered in effects related to toxicity mediated by lipid peroxidation, particularly, in cells grown in serum-free medium.

621 LIPOSOME-ASSOCIATED VITAMIN E CONFERS PROTECTION AGAINST PARAQUAT-INDUCED LUNG TOXICITY. Z Suntres and P N Shek. Operational Medicine Section, Biosciences Division, Defence and Civil Institute of Environmental Medicine, North York, Ontario, Canada. Sponsor: E M K Lui.

Paraquat is a broad-spectrum bipyridilium herbicide which causes lethal pulmonary toxicity via oxidative stress-mediated mechanisms. The rationale for the use of antioxidants in paraquat poisoning is that they act as reducing agents and free radical scavengers. Vitamin E, however, has been argued to be an ineffective therapeutic antioxidant against paraquat-induced lung toxicity. The present study was undertaken to examine whether liposome-associated Vitamin E (Lip-Vit E) can protect against paraquat-induced lung damage. Rats were administered Lip-Vit E (2 mg/kg) intratracheally and, 24 hrs later, given an i.p. injection of paraquat dichloride (20 mg/kg). Our results showed that lungs of control animals treated with paraquat for 24 and 48 hrs were extensively damaged as evidenced by significant increases in lung weight/body weight ratio and decreases in lung angiotensin converting enzyme (ACE). Also, paraquat treatment resulted in decreases in reduced glutathione (GSH) and concurrent increases in oxidized glutathione (GSSG) concentrations as well as increases in lipid peroxidation levels, suggesting oxidant stress-mediated mechanisms may be responsible for paraquat-induced lung toxicity. Pretreatment of rats with Lip-Vit E, 24 hrs prior to paraquat treatment, resulted in increases in total lung Vitamin E levels and protected the lung from paraquat-induced changes in lipid peroxidation, GSH/GSSG ratio, and lung ACE activity. Results of this study suggest that pretreatment of rats with liposome-associated Vitamin E can provide a prophylactic effect on paraquat-induced lung damage.

THE TOXICOLOGIST

An Official Publication of the

Society of Toxicology

Abstracts of the

31st Annual Meeting

Vol. 12, No. 1, February 1992