372 **THE EFFECTS OF 21-AMINOSTEROIDS ON IRON REDOX STATUS AND IRON-DEPENDENT LIPID PEROXIDATION.** T P Ryan and T W Petry. Investigative Toxicology, Upjohn Laboratories, Kalamazoo, MI.

The lipophilic 21-aminosteroids were designed as inhibitors of membrane-specific oxidations and have proven efficacious in several in vitro and in vivo models, including iron-dependent lipid peroxidation systems. In this study, the effects of two 21-aminosteroids (U-74500A and U-74006F) on iron redox behavior and iron-dependent lipid peroxidation were investigated. U-74500A completely prevented ADP-Fe(II) autoxidation whereas U-74006F had only a slight inhibitory effect. In the presence of U-74500A, formation of a Ferrozine-Fe(II) complex was slow, suggesting that this compound chelates Fe(II). In the presence of ADP-Fe(III), U-74006F and U-74500A reduced iron at rates of 0.1μM/min. and 2.0μM/min., respectively. The effects of these compounds on iron oxidation and reduction were consistent with the oxidation potentials of their amine substructures. When incorporated into rat hepatic phospholipid liposomes, U-74500A effectively inhibited iron oxidation and iron-dependent liposomal lipid peroxidation. U-74006F was minimally effective in this system. These results suggest that the antioxidant activity of U-74500A may involve effects on the redox status of iron in membranes whereas the mechanism of U-74006F action is less apparent.

373 **PROTEIN OXIDATION INDUCED BY DIQUAT (DQ) OR CCl_4: EVIDENCE FOR MULTIPLE MECHANISMS** T W Petry, D P Blakeman, R A Jolly and T P Ryan. Investigative Toxicology, Upjohn Laboratories, Kalamazoo, MI.

In addition to lipid peroxidation (LP), oxidative damage to cellular proteins may be important in oxidative injury. In studies reported here, DQ-dependent LP and protein oxidation (carbonyl formation; PC) were characterized in rat hepatic microsomal and purified cytochrome P-450 reductase/albumin systems. CCl_4-dependent LP and PC were also examined in the microsomal system. PC was quantitated by formation of 2,4-dinitrophenylhydrazone derivatives. LP was assessed as thiobarbituric acid-reactive substance (TBARS) formation. In the microsomal system, DQ induced both LP and PC formation. Near complete inhibition of LP was observed with proprietary antioxidants (U-74006F, U-78517G), while PC was only partially inhibited. With CCl_4, antioxidants resulted in near complete inhibition of both LP and PC. In the purified P-450 reductase/albumin system, DQ-dependent PC formation was similar to that observed in microsomes and was essentially unaffected by the antioxidants; minimal TBARS formation was observed. The data are consistent with diquat-dependent protein oxidation occurring by two pathways: 1)a lipid-independent pathway, which is relatively insensitive to radical-scavenging antioxidants; and 2)a lipid-dependent pathway, which is antioxidant sensitive. As would be expected, CCl_4-dependent PC formation is largely secondary to LP.

374 **DETECTION OF CARBONYL GROUPS ON OXIDIZED PROTEINS USING AN IMMUNOCHEMICAL TECHNIQUE.** N C Halmes, R J Keller, J A Hinson and N R Pumford. Division of Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR.

Chemical toxicants may produce active oxygen species that react with amino acids on proteins forming carbonyl groups and other oxidative products. An immunochemical assay was developed to detect carbonyl moieties that result from oxidative damage to proteins. Bovine serum albumin was reacted with hydroxyl radicals generated via a Fenton-like mechanism using hydrogen peroxide and vanadyl as the metal catalyst. The resulting albumin-derived carbonyls were reacted with 2,4-dinitrophenylhydrazine, giving the corresponding hydrazone. The carbonyl-dinitrophenyl groups were detected by Western blot using anti-dinitrophenyl antisera. Gels stained with Coomassie blue show a decrease in albumin intensity with increasing vanadyl concentration. The immunoblot demonstrated a concentration-dependent increase in carbonyl formation, as well as fragmentation of the albumin into two distinct bands with molecular weights of 51 kDa and 45 kDa. This indicates albumin has two sites that are highly susceptible to oxidative cleavage. In a parallel experiment, dinitrophenylhydrazone adducts were detected using an established spectrophotometric method. The spectrophotometric results were consistent with those of the Western blot, showing more carbonyl groups formed with increasing vanadyl concentration. However, this immunochemical assay was greater than three orders of magnitude more sensitive than the spectrophotometric method in the detection of oxidative damage.

375 **EFFECTS OF VARIOUS ALDEHYDES ON PLASMA ASCORBATE(ASC) LOSS AND PROTEIN OXIDATION(PO).** **C A O'Neill*, D Han+, B Tzeng+, L Packer+ and C E Cross*. Div. Pulmonary/Critical. Care, Sch. of Med.*, and Dept. Vet. Pharm/Tox#, Sch. of Vet. Med., UC Davis, and Membrane Bioenergetics Group, UC Berkeley+, CA. Sponsor: S N Giri#**

Cigarette smoke(CS) contains free-radical oxidant species including aldehydes. Human plasma, previously characterized for antioxidant defenses, was treated with various aldehydes, at concentrations representative of CS exposure, in an effort to elucidate events that occur in respiratory tract lining fluid, since these compounds may be absorbed in this fluid during CS exposure. Plasma(10ml) from normolipidemic males was treated with a mixture of 1mg acetaldehyde, 39ug propanal, 10ug butyraldehyde, 10ug isovaleraldehyde and 15ug crotonaldehyde (ALDE). An addition of 45ug(1x), 225ug(5x), and 450ug(10x) of acrolein(ACRO) was added to plasma or added(1x) to plasma with ALDE. Plasma was incubated (37°C) and aliquots drawn at 0,1,2 and 3h for parameter measurement. The ALDE group ASC level was significantly reduced at 1 and 2h(84 and 82%, respectively) compared to air control(AC). ACRO cause a dose-dependent ASC loss at 1 and 2h compared to AC(84, 70[1x]; 78,43[5x]; 74,34%[10x], respectively). ALDE and ACRO1x had a synergistic effect on ASC loss at 1 and 2h compared to AC (80 and 64%, respectively). PO, measured by protein-SH utilization, was significantly reduced with ALDE treatment to 90,87 and 84% of the AC at 1,2 and 3h, respectively. Protein-SH was reduced with ACRO in a dose-dependent manner at 1x,5x and 10x at 1,2 and 3h compared to AC(1x:94,91,90; 5x: 51,43,33; 10x:40,34,29%, respectively). ACRO1x with ALDE produced a synergistic loss of protein-SH at 1,2 and 3h to 87,84 and 81% of AC. Carbonyl formation with ALDE increased at 1, 2 and 3h to 280,246 and 287% of AC, respectively. ACRO at 1x,5x and 10x caused a dose-dependent carbonyl increase at 1,2 and 3h compared to AC(1x:210, 193, 206; 5x:710, 557, 630; 10x:1020, 825, 833%, respectively). Similarly, ALDE-ACRO1x caused a synergistic elevation in carbonyl formation compared to AC at 1,2 and 3h to 296,286 and 290%, respectively. Our data indicates ALDE and ACRO cause significant portion of plasma ASC utilization and PO damage. It can be concluded that aldehydes known to be present in CS partially mediate oxidative damage caused by this exposure.(Supported by NIHHL 47628 and 07013)