that p53 induction, apoptosis and MN formation are temporally distinct events. The early appearance of p53 protein may be involved in the activation of apoptosis in response to severe DNA damage. MN probably arise in less severely damaged cells which are able to override the G2 checkpoint. The persistence of p53 in the MNC may represent a mechanism to prevent the proliferation of these damaged cells.

494 V79 FIBROBLAST MICRONUCLEI INDUCTION BY CYSTEINE METHYL ESTER THIOL ACID ESTER CONJUGATE OF METHYLENE DIPHENYL DIISOCYANATE (MDI).

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Isocyanates are known to react with thiols, rapidly and reversibly, under physiological conditions. The biological significance of the possible formation of these thiol acid esters following exposure to diisocyanates used in polyurethane production, such as MDI, is not known. Reaction of MDI to cysteine or glutathione produced an insoluble product. A water soluble product was produced when MDI was reacted to cysteine methyl ester (CME). The resultant thiol acid ester (MDI-CME) was characterized by full NMR assignment and mass spectroscopy. Approximately one million fibroblast in the exponential phase of growth were seeded into a 100 mm culture dish and cultured over night. The medium was changed to phosphate buffer saline (PBS) and the cells were exposed for 2 hrs to 1.25, 2.5, 5 or 10 µg/ml MDI-CME/PBS. Cells were washed and incubated for 20 hrs in culture medium with the cytokinesis blocker, Cytochalasin B. Cells were then washed, fixed, stained and scored for micronuclei (MN) in binucleated cells. Vehicle (water), CME, and positive vincristine sulfate controls were run, concurrently. MDI-CME caused a dose-dependent increase in MN. Significant increases in MN were noted at ≥2.5 µg/ml. A decrease in the nuclear division index denoting loss of viability was noted at the highest dose (10 µg/ml). MDI-CME was more potent than the insoluble MDI thiol acid esters, as well as, the MDI hydrolysis product,4,4'-methylenedianaline at inducing MN. Preliminary data also suggest that MN in alveolar macrophages may be increased in mice exposed to MDI-CME by intratracheal installation. These results underscore the need for investigation of the possible in vivo formation of MDI thiol acid esters following exposure to MDI.

495 GENOTOXICITY AND INHIBITION EFFECTS OF RETINOIDS ON CELL TRANSFORMATION AND ANGIOGENESIS.

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9-*cis* retinoid acid (9-*cis* RA) and 13-*cis* retinoid acid (13-*cis* RA) are well known chemopreventive agents used in clinical trial for several years, but there are limited literature on their genotoxicity and inhibition effects on transformation and angiogenesis. In this study, 9-*cis* RA and 13-*cis* RA were tested in a rat tracheal epithelial (RTE)cell transformation assay and chorioallantoic membrane (CAM) angiogenesis assay for their inhibition effects and Ames Test and mammalian cell gene mutation (CHO AS52/XPRT assay) for their genotoxicity. For RTE assay, primary RTE cells from F344 male rats were exposed to a carcinogen, benzopyrene (B[a]P) alone, or with 5 doses of retinoids (9-*cis* RA; 30 nM - 3 µ M, and 13-*cis* RA; 0.1 - 10 nM). After 30 days in culture, carcinogen-induced transformed colonies were scored and the % reduction in transformation frequency in treated *versus* control were compared. For CAM assay, angiogenic (6Ti ras/SV*myc*) cells alone or with 5 doses of RAs (9-*cis* RA; 0.3- 30µ M, 13-*cis* RA; 0.1 to 0.001 µM), were placed on 7 day old embryonic CAM for 72 hrs, the newly formed blood vessels were captured and analyzed using a custom image analysis system. 9-*cis* RA and 13-*cis* RA inhibited the RTE transformation induced by B[a]P by 88.4 and 100 % at doses of 30 and 1 nM, respectively. 9-*cis* RA and 13-*cis* RA also inhibited the CAM angiogenesis by 69.0 and 78.0 % at doses of 3 and 0.1 µM, respectively. However, 9-*cis* RA and 13-*cis* RA did not show any genotoxicity in the Ames Test (TA98 and TA100) and AS52 / XPRT assay with and without S9 metabolic activation system at doses of 0.5 mg / plate as top dose for Ames Test and 0.03 mM as top dose for AS52 /XPRT assay. These results suggest that 9-*cis* RA and 13-*cis* RA are not only highly efficacious chemopreventive agents but also effective antiangiogenic inhibitors without genotoxicity (Supported by NCI-N01-CN-25466-06,-85142,-95017.)

496 TIME COURSE OF DNA CROSSLINK REMOVAL FOLLOWING EXPOSURE OF KERATINOCYTES TO SULFUR MUSTARD.

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Our laboratory has been studying the effects of the chemical threat alkylating agent sulfur mustard (SM) on DNA damage and repair in human cells grown in culture. The assay of choice for the past few years has been the comet or single cell gel electrophoresis assay. Initially, Moser *et al.* (SOT 1999) demonstrated that the presence of SM, a crosslinking agent, inhibited the detection of H2O2-induced single strand DNA breaks (ssb) in SM-treated human lymphocytes. We then reported (Smith *et al.*, SOT 2000) a dose response curve for this inhibition that showed the effect at concentrations of SM as low as 5 uM, and further demonstrated that the monofunctional sulfur mustard chloroethyl ethyl sulfide (CEES), which does not form crosslinks, failed to show this inhibition of ssb detection. We now report on time course experiments that suggest that the SM-induced crosslinks of human keratinocytes are markedly reduced within 18 hours after exposure and completely removed by 24 hours. The data derived from the comet assay is supported by gel electrophoretic studies and fluorometric analysis of crosslink formation. These data support the concept that DNA crosslinks are a significant part of the damage inflicted by SM and that they can be effectively removed by endogenous repair mechanisms. This information will play a critical role in the evaluation of medical countermeasures against this threat agent.

497 EVALUATION OF MATERNAL AND FETAL AH GENOTYPE ON THE ABILITY OF COAL TAR TO INDUCE DNA ADDUCTS.

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Studies have demonstrated that exposure to polycyclic aromatic hydrocarbons (PAH) in utero can potentially cause teratogenic and/or carcinogenic outcomes. The extent to which these outcomes manifest themselves depends largely on the metabolic profile of the dam and her pups, *i.e.*; their ability to metabolize PAH. This study employed the B6D2F1 x DBA backcross model to examine DNA adduct formation as a function of Ah genotype following dermal application of coal tar to pregnant dams. Coal tar (10 mg) was applied daily to the shaved backs of pregnant mice beginning on day 13 of gestation. Dams and pups were sacrificed at birth and PAH:DNA adduct formation in various tissues was evaluated using 32P-postlabeling analysis. Pup Ah genotypes were determined using Ah receptor allele-specific PCR amplification of mouse liver DNA. The DNA adduct levels in skin and lung of DBA dams were 4 and 2 times greater than the levels observed in tissues of B6D2F1 dams, respectively. In the case of pups exposed to coal tar components in utero, lung was the primary site for DNA adduct formation. DNA adduct levels were 1.4 times greater in pups of DBA dams in comparison to pups of B6D2F1 dams. A similar relationship was observed with liver DNA adduct levels. Although maternal Ah genotype appears to have influenced DNA adduct formation, there was no apparent correlation with Ah genotype of the pups. This difference is perhaps attributable to the difference in biological activity of a complex mixture *versus* that of a single PAH. This research was supported by funds from EPRI.

498 BENZO(A)PYRENE-7,8-DIHYDRODIOL (BPD)-INDUCED GENOTOXICITY IN PROSTAGLANDIN H SYNTHASE FORM-2 (PGHS-2)-EXPRESSING HUMAN CELLS.

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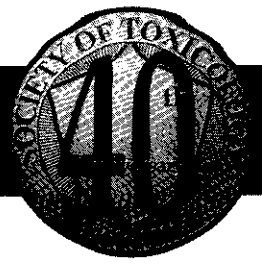
PGHS-2 catalyzes the synthesis of PG H2 from arachidonic acid (AA) and is induced by mitogens, growth factors and tumor promoters. This physiologically important reaction also causes bioactivation of promutagens and procarcinogens. Human PGHS-2 was expressed in a DNA damage repair-deficient, xeroderma pigmentosum group A (XPA) human fibroblast cells. PGHS-2-mediated genotoxicity was measured in the cells by quantitative PCR (QPCR) of 16.2 kb damage-susceptible mitochondrial DNA after treatment of the XPA cells with BPD, a known PGHS-activated promutagen and DNA damaging agent. The DNA damage assays were carried out with chemiluminescent or colorimetric assays using biotinylated primers. Levels of the PCR-replicated mitochondrial DNA fragment failed to



Society of Toxicology

40th Annual Meeting

An Official Journal of the
Society of Toxicology
Supplement

TOXICOLOGICAL SCIENCES
Formerly Fundamental and Applied Toxicology

The Toxicologist

Abstracts of the 40th Annual Meeting

Oxford University Press

Volume 60, Number 1, March 2001

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 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

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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