692 APOPTOSIS INDUCED BY INTERACTIONS BETWEEN MOLDY HOUSE MICROBES.

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Exposure to complex mixture of bacteria and fungi in moldy buildings is a potential cause of inflammatory related symptoms among the occupants. In this study, capability of these microbes to cause apoptotic cell death was investigated in more details. In addition, microbial interactions on apoptosis was identified in mouse macrophages, when the microbes were cultured separately and together on the same culture plate. The fungal strain *Stachybotrys chartarum* and the gram-positive bacterial strain *Streptomyces californicus* were selected in this study based on their characteristic occurrence in water damaged buildings. These microbes were co-cultivated on the same agar plate. The both strains were also cultured separately and the strains were combined at the same proportion as the co-cultivated combination of *S. chartarum* and *Str. californicus* (1:5). Mouse macrophages (RAW264.7) were exposed to combination of spores and spores individually. In the dose response study the macrophages were exposed to six doses (1×10^4-3×10^6 spores/ml) for 24 hours. For the time course study the macrophages were exposed to the dose of 3×10^5 spores/ml for 4, 8, 16 and 24 hours. Apoptosis was measured by flow cytometry using propidium iodide (PI) staining of permeabilized cells. Changes of caspase-3 activity in mouse macrophages were detected by fluorometric substrate cleavage assay. Flow cytometric analysis of DNA fragmentation showed, that there was 2.6±0.1 % apoptotic cells when exposed (3×10^5 spores/ml at 24 hr) to separately cultured combination, but the amount of apoptotic cells increased to 12.4±1.3 % when exposed to co-cultivated combination. Furthermore co-cultivation increased the caspase-3 activity by 2.4-fold compared to separately cultured combination. Altogether, this data suggests that co-culture of *S. chartarum* and *Str. californicus* leads to microbial interactions, which significantly affect on the ability of microbes' spores to cause apoptosis in mammalian cells.

693 DIFFERENCES IN THE ONSET OF APOPTOSIS IN OLFACTORY SENSORY CELLS OF MICE, RATS AND MONKEYS GIVEN AN INTRAVENOUS INJECTION OF MAXIMUM TOLERATED DOSE (MTD) OF VINCRISTINE, A VINCA-ALKALOID ANTITUMOR DRUG.

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We have previously reported that vincristine sulfate (VCR) causes apoptosis in the olfactory sensory cells of male mice following an intravenous injection. In the present study, we investigated differences in the onset of apoptosis in the olfactory epithelium of BALB/c mice and Crj:CD(SD)IGS rats given an intravenous injection of VCR at a MTD and estimated 10% lethal dose (LD10), and of common marmoset monkeys receiving MTD. Namely, VCR was intravenously administered once to mice (1.17 and 1.95 mg/kg), rats (0.21 and 0.35 mg/kg) and monkeys (0.35 mg/kg) of both sexes. The dosing day was designated as Day 1 in this study. The animals were serially necropsied on Days 2, 5 and 10, and the nasal tissue and sciatic nerve were examined light-microscopically. The olfactory sections of mice on Day 2 were subjected to the terminal deoxyribonucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling (TUNEL) assay and electron-microscopic observation. In mice, VCR elicited cell deaths with condensation and fragmentation of nuclei in the olfactory epithelia and vomeronasal organs in males given 1.95 mg/kg and in females receiving 1.17 mg/kg or more from Day 2. On Day 10, demyelination in the sciatic nerve was noted in both sexes at 1.17 mg/kg or more. TUNEL-positive cells were also recognized in the basolateral olfactory epithelium. Ultrastructurally, condensation and margination of chromatin were observed in sensory cells of the basolateral olfactory epithelium. However, no morphological changes related to VCR treatment were observed in rats and monkeys of either sex. In conclusion, these results demonstrate that MTD of VCR evokes severe olfactory apoptosis only in mice, especially in females.

694 EFFECTS OF STAINLESS STEEL MANUAL METAL ARC WELDING FUMES ON DNA DAMAGE AND APOPTOSIS INDUCTION *IN VITRO* AND *IN VIVO*.

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Epidemiological studies examining the potential association of occupational inhalation of welding fumes and increased incidence of lung cancer have not reached definite conclusions. Stainless steel (SS) welding fumes are of particular interest because they contain potential human carcinogens, such as chromium and nickel. Animal studies addressing toxicological responses related to carcinogenicity are currently lacking, although SS welding fumes have been shown to be mutagenic *in vitro*. The goals of this study were to examine the potential for SS welding fumes to damage DNA and induce apoptosis, events that are associated with possible carcinogenic activity. In a previous study using electron spin resonance, the generation of hydroxyl radicals from Cr(VI) in the SS fume (1.0 mg/ml) was observed. In the current study, SS fume at the same concentration caused plasmid DNA strand breakage *in vitro* under similar conditions. To examine the effects of SS fumes on apoptosis *in vivo*, male Sprague-Dawley rats were intratracheally instilled with SS fumes from manual metal arc welding (1.0 mg/100 g bw), Cr(VI) (0.2 mg/100 g bw) as a positive control, or the saline vehicle. On days 1, 3, 6, 8, and 10, the rats were euthanized and their left lungs were excised and cryo-sectioned. Apoptosis was examined in the tissue sections by TUNEL assay. Increased numbers of apoptotic cells were found in lung tissue treated with either the SS fume or the positive control, Cr(VI), as compared to lungs from saline-treated animals. These preliminary studies indicate that SS welding fumes can damage DNA *in vitro* and induce apoptosis *in vivo*. Studies are ongoing to determine if the apoptotic event is associated with an increase in oxidative DNA damage in lung tissue.

695 INHALED OZONE INDUCES DNA-DNA CROSS-LINKING IN EXPOSED RAT LUNG.

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Ozone (03) exposure has been shown to cause a variety of debilitating respiratory disturbances including possibly cancer. The prevalence of 03 as the dominant oxidant of world-wide photochemical smog, its occupational utilization and its application as a direct or adjunct therapy for treatment of various diseases make it a global human health concern. As the major target of 03 inhalation, lung tissue has been reported to sustain both cytotoxic and genotoxic damage as well as cause neoplastic transformation *in vivo* and *in vitro*. However, whether exposure to O3 can cause DNA-DNA cross-linking is as of yet unknown. Thus, this study examined whether long-term /chronic 03 exposure could induce pulmonary genomic DNA-DNA cross-linking. Adult male rats were exposed nose-only, 5h/d, 5d/wk for 4, 8, 12, 24 and 48 wks to filtered air or one of three different O3 concentrations (i.e., 0.1, 0.3 and 0.6 ppm). Rats were sacrificed 3d after the cessation of exposure lungs were removed and flash frozen and DNA-DNA cross-links determined by gel electrophoresis. Effects of 03 appeared to be time- and dose- dependent. Animals exposed to 0.3 ppm 03 for 8 wks demonstrated significantly elevated levels of cross-linking compared to controls and 0.1 and 0.6 ppm 03 exposed rats; exposure for twelve wks to 0.3 and 0.6 ppm 03 resulted in greater DNA cross-linking than controls or the 0.1 ppm 03 group; inhalation exposure of rats for twenty four wks to all three concentrations caused a significant dose-related increase in cross-linking; at 48 wks rats exposed to 0.3 ppm O3 demonstrated significantly elevated levels of cross-linking (compared to air controls). These findings suggest that long-term inhalation of 03 can produce genotoxic events that could be associated with neoplastic changes. NIOSH # OH03607.

696 ROLE OF INDUCIBLE NITRIC OXIDE-DERIVED NITRIC OXIDE IN SILICA-INDUCED PULMONARY INFLAMMATION AND INJURY.

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Our lab has demonstrated previously that exposure of rats to silica resulted in induction of inducible nitric oxide synthase (iNOS) in alveolar macrophages (AM) and alveolar type II epithelial cells (TII). The production of nitric oxide (NO) by these pneumocytes and resultant NO-dependent damage (nitrotyrosine residues) have been related temporally and anatomically with silica-induced inflammation and granuloma formation (Am J Physiol Lung Cell Mol Physiol 283:L485, 2002). The objective of the present study was to determine if these associations represent a causal role for NO in silica-induced lung disease. To address this question, the subchronic response of C57BL/6J wild type (WT) mice and iNOS knockout (KO) mice to aspiration of silica (40 mg/kg) was compared 42 days post-exposure. Exposure of WT mice to silica was marked by the following sub-chronic responses: pulmonary damage (increased LDH and albumin levels in lung lavage fluid, and increased lung weight), inflammation (increased lung lavage polymorphonuclear leukocytes, TNF-alpha, MIP-2 and TGF-beta, and histological evidence of alveolitis and lipoproteinosis), oxidant stress (increased zymosan-stimulated chemiluminescence from AM, and decreased total antioxidant levels in lung lavage fluid), and

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