2508

PULMONARY CLEARANCE OF WELDING FUME PARTICLES AS ASSESSED BY MAGNETOMETRY AND CONFOCAL MICROSCOPY.

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Pulmonary responses to welding exposures may vary due to the materials used during welding. Welding fume was collected during shielded electric arc welding from two types of consummable electrode wire: stainless steel (SS) and mild steel (MS). The objectives were to analyze the composition of the samples, describe the pulmonary clearance kinetics of the two fumes, and develop methods for imaging fume particles in the lungs. Bulk analysis of the metal constituents of the fume was characterized by energy dispersive spectroscopy (SS: 52.3% Fe, 22.2% Cr, 18.3 % Mn, 4.9% Ni, 2.3% Si; MS: 89.2% Fe, 8.2% Mn, 2.6% Si). CD/VAF rats (n=4) were intratracheally instilled (1 mg/100 g b wt) with the SS or MS welding fume samples suspended in sterile saline. At 1, 7, and 14 days post-instillation, the lungs were removed. Due to the magnetic nature of the welding fumes, the quantity of particles present in the lungs could be assessed by magnetometry. The MS welding fume was eliminated from the lungs faster ($p<0.05$) than the SS welding fume. By 14 days, 35.5% of the initial dose of the MS particles but only 7.7% of the SS particles had been cleared from the lung. Images of particles in lung tissue were generated by confocal microscopy of aldehyde-fixed, fluorescent-stained (Lucifer Yellow CH; 0.1 mg/ml) lung pieces embedded in Spurr's epoxy. Images were recorded from a laser scanning confocal microscope fitted with an argon-ion laser using the 488 nm excitation light. With a fluorescent emission spectra >510 nm, lung tissue and cells were imaged. When using polarized light <510 nm simultaneously passed to a separate optical path, images of welding fume in the lungs were recorded which were suitable for quantitation. We have developed a method to image particles in lungs and have shown that welding fumes of different composition are cleared at different rates. (American Welding Society and NIOSH 109979).

2509

EFFECTS OF SILICA EXPOSURE ON SUBSTANCE P IMMUNOREACTIVITY AND PREPROTACKYKININ MRNA EXPRESSION IN TRIGEMINAL SENSORY NEURONS.

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Trigeminal sensory neurons innervate the nasal cavity and may release substance P (SP) upon exposure to inhalated irritants. The purpose of this study was to determine if silica dust, an occupational irritant causing inflammation, activates sensory neurons supplying the nasal cavity. Rats were placed in inhalation chambers and exposed daily to 2mg/m^3 of fresh silica (<5um) or filtered air for six months (n=3). The trigeminal ganglia (TG) were removed and prepared for SP immunocytochemistry and preprotackykinin (PPT) *in situ* hybridization. SP-like immunofluorescence in TG neurons was categorized as high, moderate, or low intensity. *In situ* hybridization autoradiographs were quantified on the basis of grain density using digital imaging analysis. The SP immunoreactivity and PPT mRNA expression in the TG neurons were significantly increased after silica inhalation. The proportion of highly positive SP-immunoreactive neurons shifted from 1.30±.58% in controls to 11.30±1.15% after silica treatment. The neurons exhibiting high grain density increased from 1.50±.87% in controls to 11.67%±.58 in the silica group. Thus, inhalation of silica results in increased levels of immunoreactive neuronal SP and PPT mRNA. These findings suggest that silica activates sensory pathways which may be involved in nasal inflammation.(Support by BOM Grant #G1125142/54A7).

2510

RATS EXPOSED TO CIGARETTE SMOKE HAVE ALTERATIONS IN LUNG PROTEOGLYCAN COMPOSITION

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Proteoglycans (PGs) are polyanionic substances that are present in the lung within cells, on cell surfaces and in the extracellular matrix. We theorized that lung content and distribution of these compounds may be changed by cigarette smoke. Sprague Dawley rats were randomly divided into five groups (n=6 in each group).Three groups were exposed to cigarette smoke for 5 (Grp1), 10 (Grp2), or 20 (Grp3) consecutive days. The other two groups (Grp4,Grp5) were controls. Lung parenchyma, airways and pulmonary artery were partitioned and $^{35}[Na_2SO_4]$ labelled PGs were analyzed by cellulose acetate plate electrophoresis. Lung PG content (mg of PG/ g wet tissue) was highest in Grp2 rats (63.24 mg/g). Airway chondrotin sulfate (CS) levels was highest in Grps 1 and 3; Grp2 levels were similar to baseline control values. Airway heparin (HP) levels increased 15 fold after 5 days of smoke exposure and returned to baseline by 10 and 20 days of smoke exposure. Pulmonary artery CS and HP levels decreased in all smoke exposed groups. Immunostaining of lungs with anti - HP and anti - CS antibodies showed a marked decrease in HP staining of the alveolar basement membranes in Grps 1 and 3 compared to the other groups. CS staining was not altered. Histopathology showed increased cellularity and matrix deposition in Grp 1 rats and a predominance of perivascular mononuclear and polymorphonuclear cells in Grp 2 rats. These data suggest that cigarette smoke inhalation induces changes in lung proteoglycan balance. These fluctuations in PG levels may contribute to the spectrum of lung injury seen with cigarette smoke.* Supported by HL-36829

2511

PULMONARY FIBROSIS AND CYTOKINES: EXPRESSION OF a-SM ACTIN BY ALVEOLAR MYOFIBROBLASTS.

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In normal lungs, fibroblastic cells in alveolar septa contain cytoplasmic stress fibers but do not express a-smooth muscle (a-SM) actin. In fibrotic conditions, such as in Bleomycin induced experimental fibrosis in the rat and in Idiopathic Pulmonary Fibrosis these cells are transformed into typical myofibroblasts (MF) which express a-SM actin and then, as shown in the Bleomycin model, synthesize collagen (ZHANG et al. Am.J.Pathol., 1994, 145:114). According to our studies and others, replication of MF and a-SM actin synthesis by them are mediated by cytokines such as TGFb1 and TFNa. These cytokines are stored in- and in part synthesized by-regenerating type II epithelial cells and by alveolar macrophages. Moreover, intratracheal instillation of GM-CSF is followed by a-SM actin expression by alveolar MF, and potentiates pulmonary fibrosis due to intratracheal Bleomycin in the rat. It appears hence, that TGFb1 and TNFa stored in regenerating type II epithelial cells, as well as GM-CSF, mediate replication of fibroblastic interstitial cells, which are transformed into a-SM actin laden MF which in turn synthesize collagen, thus producing pulmonary fibrosis. (Supported by Swiss National Science Foundaion Grant N° 40372.94)

2512

COMPARISON OF INTEGRIN αEβ7 EXPRESSION AND TNF-α PRODUCTION IN TWO MODELS OF BLEOMYCIN-INDUCED PULMONARY FIBROSIS IN MICE.

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Lung injury is accompanied by inflammation and subsequent fibrosis in bleomycin (BLM) mouse model of pulmonary fibrosis. We investigated integrin α Eβ7 expression on bronchoalveolar lavage (BAL) T lymphocytes and TNF-α production in two BLM models of pulmonary fibrosis: intratracheal (I.T., single dose) and subcutaneous (sc., multidose) injection. The dominant T lymphocytes infiltrating the lung after BLM injection were CD4$^+$ cells. In the single dose model 37% of them expressed αEβ7 at day 2 and thereafter the proportion of αEβ7$^+$ cells decreased to 11% at day 22. Of the CD8$^+$ cells 4% expressed αEβ7 at day 2. However, the proportion of αEβ7$^+$ cells increased to 68% at day 22. In the multidose model, about 30% of the CD4$^+$ expressed αEβ7 with no changes throughout the experiment. The percentage of CD8$^+$ T cells expressing αEβ7 changed from 30% at day 7 to 1% at day 21 and increased thereafter to 44% at day 49. More than 80% of the γδ T cell population expressed αEβ7 at all time points with no differences between the two models. Moreover, in both models the expression of αEβ7 was found to be much higher on γδ T cells compared to CD4$^+$ and CD8$^+$ cells. After I.T. instillation of BLM the level of TNF-α showed a single peak between days 4 and 7. In the multidose model the level of TNF-α in BAL fluid increased steadily starting after 2 of the 8 s.c. injections of BLM. Analysis of intracellular TNF-α revealed that γδ T cells are a potential source for this cytokine. These results suggest that intraepithelial (αEβ7$^+$) γδ T cells are able to produce TNF-α and may in part be responsible for the development of pulmonary fibrosis after BLM instillation. (supported by NHLBI grant 2 ROI HL27354)

2513

DOWN-REGULATION OF COLLAGEN GENE EXPRESSION BY TAURINE AND NIACIN IN THE BLEOMYCIN-HAMSTER MODEL OF LUNG FIBROSIS.

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Taurine and niacin have been previously found to block the accumulation of collagen in lung in the multidose bleomycin (BL) hamster model of pulmonary fibrosis(PF). This study was designed to evaluate if taurine and niacin would block the increases in procollagen I and III mRNA levels in the same model of PF. Hamsters were intratracheally (IT) instilled with three consecutive doses of saline or BL at weekly intervals (2.5, 2,1.5 units/5ml/kg). Animals were fed diet containing either 2.5% taurine and 2.5% niacin or the same diet without the drugs throughout the experiment. The four groups were saline-instilled with the same diet (SCD), saline-instilled with taurine-niacin in diet (STN), BL-instilled with control diet (BCD), and BL-instilled with taurine-niacin in diet (BTN). Steady-state transcript levels in the total RNA prepared from lungs of all 4 groups were determined at 0,3,7,14 and 21 days after the BL-instillation by slot blot and Northern blot analyses. Results indicated that the procollagen I mRNA level was elevated as compared to saline control by 1.5, 2.25 1.75, 1.6,1.5 fold at 0,3,7,14, and 21 days after the last IT instillation, respectively. In vivo treatment with taurine and niacin decreased the steady state level of BL-induced increase of procollagen I mRNA gradually from day 0 through 21, however, it showed maximal inhibition at day 21. We observed a similar pattern of procollagen III inhibition by combined treatment with taurine and niacin and it decreased the abundance of this mRNA from day 7 through day 21. A nuclear run-on assay is under investigation which would shed light on the down-regulation of collagen gene expression either at transcriptional or at post transcriptional level. Total and differential cell counts of bronchoalveolar lavage cell(s) (BALC) showed no significant differences between SCD and STN control groups. In contrast, the total cell population was significantly higher in the BCD group than BTN group. (Supported by NHLBI#5RO1 HL27354).

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ABSTRACTS

PART I

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