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MUTAGENICITY OF DIESEL EXHAUST SOOT DISPERSED IN PHOSPHOLIPID SURFACTANTS

Wallace, W., M. Keane, S. Xing, J. Harrison, M. Gautam, T. Ong
Division of Respiratory Disease Studies, National Institute for
Occupational Safety and Health, and West Virginia University, Morgantown,
West Virginia 26505, U.S.A.

INTRODUCTION

Organics extractable from respirable diesel exhaust soot particles by organic solvents have been known for some time to be direct acting frameshift mutagens in the Ames Salmonella typhimurium histidine reversion assay (Huisingh, et al). Upon deposition in a pulmonary alveolus or respiratory bronchiole, respirable diesel soot particles will contact first the hypophase which is coated by and laden with surfactants. To model interactions of soot and pulmonary surfactant, we dispersed soots in vitro in the primary phospholipid pulmonary surfactant dipalmitoyl glycerophosphorylcholine (lecithin) (DPL) in physiological saline. We have shown that diesel soots dispersed in lecithin surfactant can express mutagenic activity, in the Ames assay system using S. typhimurium TA98, comparable to that expressed by equal amounts of soot extracted by dichloromethane/ dimethylsulfoxide (DCM/DMSO) (Wallace, et al, 1987). Here we report additional data on the same system using additional exhaust soots and also using two other phospholipids, dipalmitoyl glycerophosphoryl ethanolamine (DPPE), and dipalmitoyl phosphatidic acid (DPPA), with different ionic character hydrophilic moieties. A preliminary study of the surfactant dispersed soot in an eucaryotic cell test system also is reported.

MATERIALS AND METHODS

Soots D4 and D6 were collected at two different times as scrapings from the exhaust pipe of a diesel tractor-trailer immediately after the engine was stopped. Surfactant dispersions were made by ultrasonic probe sonicating 25 mg of commercially obtained DPL, DPPE, or DPPA into 10 ml of 0.85% physiological sterile saline (PSS) for 10 min at 40 watts power. For surfactant dispersions, 2.5 mg soot was mixed into 2.5 ml of the appropriate phospholipid dispersion. Mixtures were sonicated by 40 watts sonicator probe for one min, then incubated in the sonicator water bath for 30 min at room temperature; the mixture was then incubated for one hour at 37C while being rotated continuously. Dilution series were made with saline. For solvent extraction, 2.5 mg soot was mixed into 2.5 ml of DCM and incubated in a sonicator water bath for 30 min at room temperature. The sample was then mixed with 2.7 ml DMSO, placed in a dry bath at 45C, and the DCM evaporated under a stream of nitrogen gas until 2.5 ml of liquid remained. Ames salmonella/microsome assay utilizing TA98 for frameshift mutation was used for mutagenicity testing, with and without S9 activation, as described by Ames et al (1975). Unfiltered sample extractions or dispersions were preincubated with tester cells for 90 min at 37C : 0.1 ml of sample was mixed with 0.5 ml of PSS, or of S9 mixture, and 0.1 ml of an overnight TA98 culture. Incubated samples were then added to 2 ml of top agar supplemented with biotin and a trace amount of histidine, and overlayed onto Vogel-Bonner minimal media plates. After 48 hours incubation at 37C, revertant colonies were counted. Solvent controls

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were DMSO, DPL, DPPE, DPPA, and saline. Positive controls were trinitrofluorenone (1 ug/plate), for samples not treated with S9, and 2-aminoanthracene for S9 treated samples (2.5 ug/plate). All samples were assayed in duplicate. Sister chromatid exchange (SCE) assay was performed, following Latt, et al (1981), using V79 cells, of Chinese hamster pulmonary fibroblast origin. Stock diesel sample suspensions were made as 10 mg soot/ml DPL suspension, or 20 mg soot/ml in DMSO. Aliquots of these soot preparations were added to 15 ml of culture medium per sample flask to result in concentrations of 25, 50, and 75 ug soot/ml medium. After 12 hours incubation, 200 ul of 25 uM 5-bromodeoxyuridine (BrdU) was added to each flask. After another 35 hours incubation, 200 ul of 10 ug/ml colcemid were added; and the cells were harvested three hours later. Cells were swollen by hypotonic saline, and fixed twice with methanol/acetic acid, and burst on microscope slides. Cells on the slide were stained with Hoechst 33258, exposed to UV light in 56C Sorenson's buffer, and then stained with 7% Giemsa. A total of 25 complete M2 chromosome spreads per slide were scored for SCE. Mitotic indices were calculated from 100 consecutive chromosome spreads per slide. Student's t-tests were used to evaluate significance of differences between samples, with p 0.05.

RESULTS

Figures 1. a. and b. show the mutagenic activities, assayed in the Ames test system with and without S9 activation, of soots D4 and D6 respectively, dispersed in the phospholipid surfactants DPL, DPPE, or DPPA, or dispersed in saline alone, or in DCM/DMSO. Phospholipid dispersed samples and equal amounts of soot extracted with DCM/DMSO are seen to express comparable mutagenic activity in the absence of S9 activation.

Figure 1. Mutagenic activity versus dose of diesel soots (a.) D4 and (b.) D6 in the Ames test system, with and without S9 activation. Samples were prepared in dichloromethane/ dimethylsulfoxide (M label), in saline (S), in DPL/saline (L), in DPPE/saline (E), or in DPPA/saline (A).

The activities after S9 activation show a diminution of the phospholipid prepared samples in comparison with the DCM/DMSO prepared samples: for D4 only the DCM/DMSO and DPL prepared samples show unequivocal mutagenic activity; and for D6 only the DCM/DMSO prepared sample expresses mutagenic activity. Figure 2. shows the activity administered at a dose level of 0.1

mg/ plate, for whole dispersion and for filtrate of the dispersion when filtered through an 0.2 micron teflon filter. For most of the DCM/DMSO prepared samples, the activity is found in the filtrate. No activity above background is expressed by the surfactant preparation filtrates. Figure 3. shows the SCE activity of soot D4 in DMSO and in DPL. Mean values and standard errors are shown for the number of SCEs in each of 25 complete chromosome spreads for four dose levels in both DPL and DMSO preparations, and for controls containing no soot. Criteria for positive response are met for both preparations for doses of 50 ug/ml and above. When the DPL dispersion of soot D4 was filtered through an 0.2 um pore filter and this filtrate was then assayed for SCE activity, the criteria for positive response was not met at any of the doses tested.

Figure 2. Mutagenic activity of total dispersion or of the filtrate of soot D6 in solvent and surfactant preparations, using TA98 without S9. Figure 3. Sister chromatid exchange frequencies in V79 cells after treatment with diesel soot D4 in DMSO or in DPL/saline. Standard errors are shown as bars.

DISCUSSION

While the surfactant extract of soot does not express mutagenic activity, the surfactant dispersion of soot does. The distinguishing factor here is the use of the unfiltered dispersion, rather than its filtrate as used in studies which have reported little or no activity for pulmonary surfactant- or lecithin-in-saline extract of diesel soot (Brooks, et al, 1984; King, et al, 1981). This suggests that the mutagenic activity is carried as a surfactant solubilized (dispersed) particulate material rather than as an extracted (dissolved) fraction in the DPL preparations. Preparation with the two other surfactants results in roughly equal activity to that expressed in the DPL preparation. It appears that the electrophilic trimethylammonium or amine moieties do not appreciably alter the mutagenic activity of the soots in comparison with the dipalmitoyl phosphatidic acid surfactant preparation. Diminution of diesel soot activity following incubation with S9 is a known effect for conventional solvent extraction preparations; but we additionally observe a decrease in the ratio of activities of DPL to DCM/DMSO prepared samples following preincubation with S9. A number of possible mechanisms responsible for this can be speculated and require investigation: S9 enzymatic activity might alter the lipid coating on soot particles, diminishing their solubilization, uptake, or activity; lipid coating on soot particles might facilitate S9 association

and detoxification of direct acting mutagens on soot surfaces; organics in the S9 preparation might adsorb to the lipid/soot surface providing a temporary prophylactic coating on the particles. Our preliminary data on eukaryotic cell SCE response to a DPL dispersed soot suggest surfactant coated and solubilized small respirable sized particles may be able to express their genotoxic activity in eukaryotic cells. Therefore, pulmonary surfactant may provide a mode of transport of respired insoluble particulate genotoxic activity in the acinar regions of the lung. The use of surfactant dispersed samples for genotoxicity testing in eucaryotic cell systems might retain complex particle structures, such as organics adsorbed on mineral dusts, to the degree they are retained upon deposition in the lung, and suppress interfering prompt particle cytotoxic effects to the same degree. We are developing a test stand to provide different modes of association of diesel exhaust organics with respirable mineral particles to generate complex particles for such tests. General questions remain as to how surfactant dispersed soots express genotoxic activity, and as to what particle properties and cellular interactions affect that activity. These include issues of particle size constraints on uptake through procaryotic cells wall and eucaryotic cell and nucleus membrane barriers; and of lysosomal enzyme digestion in addition to microsomal enzymatic activation and biotransformation of particulate genotoxicants.

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