RESPONSES OF CULTURED LUNG EPITHELIAL CELLS TO AIRBORNE DIESEL EMISSIONS

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A method for evaluating cells exposed in real time to airborne engine emissions is desired as a more rapid and physiological alternative to exposing cells to culture medium treated with collected emission samples. Such a method is especially needed for evaluating the toxicity of nanoparticles of condensed organic material that may no longer be in particulate form when contact with cells is actually made. Alveolar type II cells are the metabolically active cells of the deep lung and are potentially important targets of inhaled materials. In the intact lung, these cells are exposed to gases, vapors, and particles with only a thin layer of epithelial lining fluid between the cells and the atmosphere. In conventional tissue culture, cells are grown on a solid matrix and submerged much more deeply in tissue culture medium. Interaction of atmospheric components with the tissue culture medium may alter the effects of these components on the cells, either by dissolution of particle-associated materials in the medium, phase separation of hydrophobic components, or reaction of chemicals in the exposure atmosphere with constituents of the medium. Assessing responses of cultured type II cells to airborne materials may therefore provide a more physiologically relevant method for rapid screening of the effects of modifications of engine technology.

To mimic more closely the physiological environment, we cultured A549 cells (a type II-like human cell line) on microporous membranes (Falcon Transwells ®), which were then placed in a recently developed exposure apparatus (Cultex®) with culture medium provided only at the basolateral surface. Temperature was controlled by circulating 37°C water around the glass compartments containing the medium and around the atmosphere delivery ports. The apical surface of the cells was then exposed to exhaust from a 2000 model Cummins 5.9 L B series engine burning current U.S. certification fuel and operated on repeating heavy-duty certification duty cycles. Exhaust was diluted to 1000 $\mu g/m^3$ total particulate material and passed over the cell surface at a flow rate of 8.3 ml/minute per Transwell. Cells were exposed either to whole exhaust, to filtered exhaust, or to clean air as a control. Cells were exposed for 1 or 3 hours, then medium was restored to the apical surface and the responses were assessed after approximately 1 or 24 hours further incubation. Analyzed responses included lactate dehydrogenase release and ATP content as measures of overt toxicity; cleavage of a water soluble tetrazolium salt as a measure of cell numbers and metabolic activity; release of Interleukin-8 as a marker of inflammatory potential (after 24 hr incubation only); cellular content of total or reduced glutathione as a measure of oxidative stress; and permeability of the monolayer to Evans Blue-labeled bovine serum albumin as an indicator of epithelial integrity.

These preliminary experiments indicated no overt cytotoxicity or alterations in cell number or metabolic activity due to the exhaust. However, both whole and filtered exhaust caused a substantial increase in epithelial permeability compared with exposure to filtered air. Glutathione content was reduced by exposure to diesel exhaust. Exposure to the filtered exhaust, but not exposure to exhaust containing particulate material, increased the level of interleukin-8 detected in the conditioned medium. These encouraging preliminary results indicate that this exposure technique provides a useful method for detecting changes in the function of type II-like cells exposed to diesel exhaust and other complex atmospheres, and suggest that alterations in pro-inflammatory potential and changes in cell-cell contact may be involved in the toxicity of these materials when inhaled. Exposures at lower concentrations and to specific fractions of exhaust will follow.