COMPARISONS OF IN VITRO CYTOTOXICITY RESULTS WITH IN VIVO PULMONARY EFFECTS FOLLOWING INHALATION EXPOSURES TO A VARIETY OF MINERAL DUSTS: HOW WELL DO THEY COMPARE?

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INTRODUCTION

In vitro toxicity assays have been proposed as substitutes for acute in vivo studies during the early phases of inhalation toxicology testing. Short-term toxicity tests are needed to screen materials. Test systems generally are comprised of cultured lung cells and dusts. In vitro tests have strengths as well as limitations. These assays could be utilized in toxicology screening strategies and have the advantage of being simpler, faster and less expensive than their in vivo counterparts. In contrast, many determinants of dust toxicity are difficult or impossible to evaluate with cell cultures alone. The studies presented here were undertaken to compare in vitro results using lung epithelial L2 cells with a variety of particle-types which had been previously tested in an inhalation bioassay. Lung epithelial (L2) cells were incubated at various concentrations with either carbonyl iron (CI), zinc oxide (ZnO), crystalline silica (Si) or titanium dioxide (TiO$_2$) particles. Cytotoxicity was assessed using the MTT assay, as well as measuring for lactate dehydrogenase activity in the cell culture fluid. The results obtained in the in vitro assays were then compared to bronchoalveolar lavage fluid biomarkers of toxicity measured following 3-day inhalation exposures to each of the particulate materials described above. The results of in vitro studies indicated that titanium dioxide particles were the least toxic, carbonyl iron and silica were intermediate, and zinc
oxide particles produced the greatest toxicity. In contrast, the results of inhalation tests indicated that carbonyl iron and titanium dioxide produced little or no pulmonary effects. Silica produced a progressive pulmonary lesion characterized by inflammation, Type II epithelial cell cytotoxicity and consequent granulomatous formation. Inhalation of zinc oxide caused a potent but transient pulmonary inflammatory response, which resolved within a few days postexposure, due, in part, to its high solubility in the lung. The results with zinc oxide and carbonyl iron particles point out the limitations of the *in vitro* tests. The *in vitro* toxicity test does not provide for pulmonary clearance of exposed dusts, either by solubility (in the case of zinc oxide) or by macrophage phagocytosis (as was evident in the lungs of carbonyl iron-exposed rats). Perhaps a sophisticated co-culture system (e.g., epithelial cells and macrophages) would be more useful in predicting the fibrogenic potential of inhaled dusts.

**METHODS**

**In Vitro Studies**

Rat lung epithelial cell lines (Rat L2 cells) were purchased from American Type Culture Collection (Rockville, MD) and grown to log phase using Kaighn’s Nutrient Mixture F-12 (Ham’s F-12) (10% FBS + concentrated glutamine + antibiotic-antimycotic solution) at 37°C, 5% CO₂. Following passage from culture flasks, L2 cells were added to designated wells (1 x 10⁵/well in 24 well plates (Corning Glass Works, Corning, NY; diameter = 16 mm) for LDH determination or 1 x 10⁴/well in a 96 well plate (diameter 4.5 mm) for MTT determination plated at 1250 cells/mm² for the MTT assay and plated at 1000 cells/mm² for LDH analysis. Cells were allowed to adhere overnight in wells at 37°C and 5% CO₂. During the next day, autoclaved silica, zinc oxide, titanium dioxide, and carbonyl iron particles were added to