CHANGES IN F-ACTIN ORGANIZATION INDUCED BY HARD METAL PARTICLE EXPOSURE IN RAT PULMONARY EPITHELIAL CELLS AS OBSERVED BY LASER SCANNING CONFOCAL MICROSCOPY

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Hard metal is an alloy of tungsten carbide and cobalt along with other components such as chromium carbide, molybdenum carbide, tantalum carbide, and nickel. Chronic exposure to hard metal particles by inhalation causes alveolitis leading to interstitial fibrosis, the pathogenesis of which is still undefined. The initial inflammatory response includes a change in epithelial cell permeability barrier function (1) which has been shown to be regulated by the state of assembly and organization of the actin cytoskeletal network (2, 3). Therefore, the objective of this study was to evaluate the effect hard metal particles have on F-actin organization of rat lung epithelial cells in an *in vitro* culture system.

Rat lung epithelial cells (L2: ATCC, CCL-149) were grown to confluence on glass coverslips and exposed to various concentrations of hard metal particles for 24 hours. The effect on F-actin organization was visualized by confocal microscopy following Bodipy-Phallacidin staining, while changes in cell morphology were assessed by phase contrast microscopy. Hard metal particles of cobalt, tungsten carbide, and tungsten carbide/cobalt (6 % cobalt) were tested at concentrations of 1, 3, and 5 μg/ml. There was a dose-dependent change in the F-actin organization in the cells. The actin microfilaments lost their uniform distribution and aggregated into homogeneous masses of F-actin staining. Significant change in F-actin state was observed even at a 1 μg/ml concentration of tungsten carbide/cobalt particles. This is consistent with previous observations that pathological effects of tungsten carbide/cobalt particles are more pronounced compared to either metal alone. Phase contrast microscopy revealed no significant change in the cell morphology at this short incubation time.

In view of the abundance of evidence for the role of F-actin in epithelial cell permeability barrier function, we conclude the inflammatory response evoked in the lung by inhaled hard metal particles may be, at least partly, due to alteration in the actin cytoskeleton of lung epithelial cells. These changes observed in the cytoskeletal network may be the basis for the pathogenesis of hard metal-induced lung disease.

References

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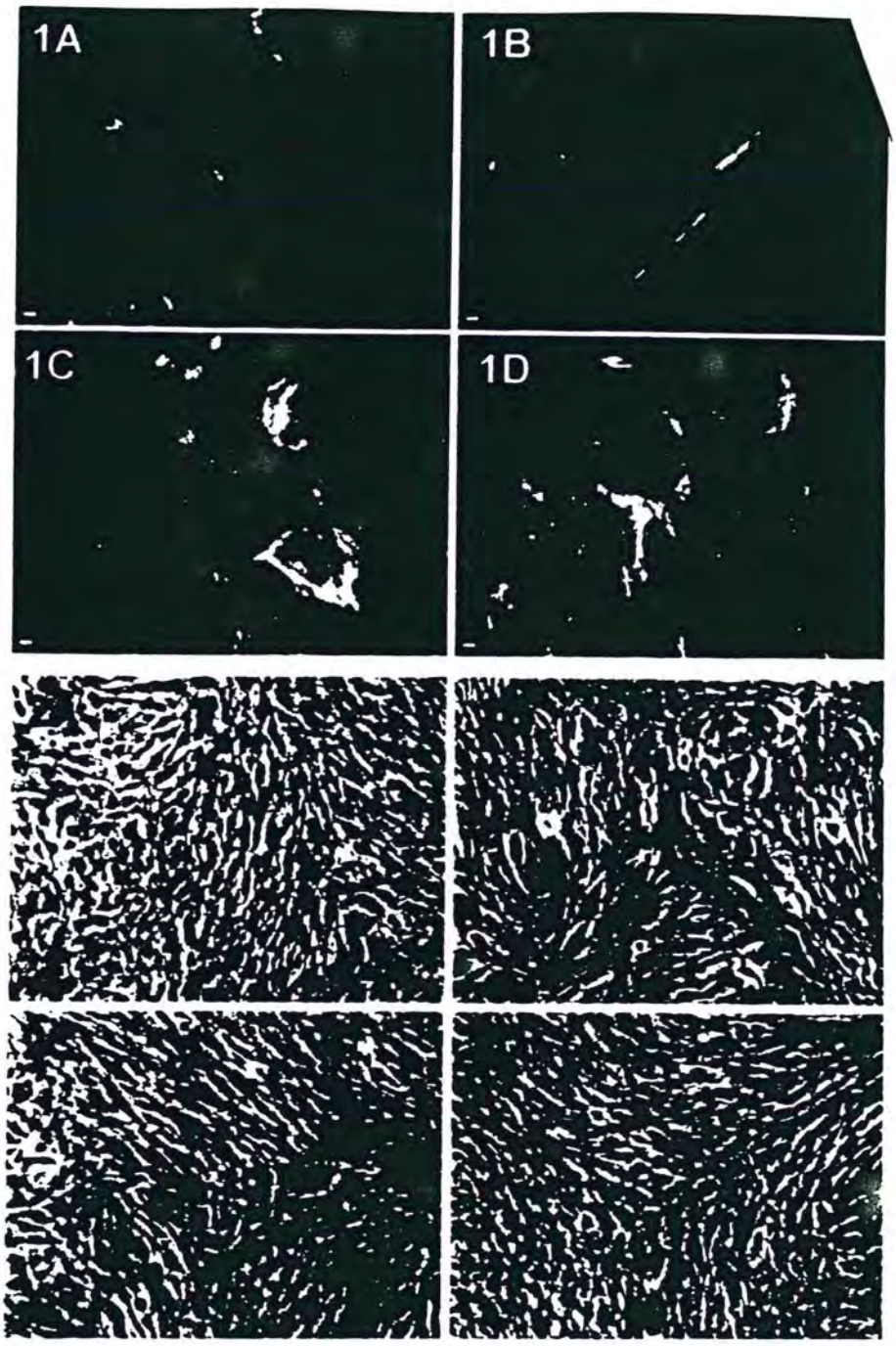

FIG. 1. Confocal micrographs of rat lung epithelial cells exposed to 1 μg/ml of hard metal particles: (A) control; (B) tungsten; (C) tungsten carbide/cobalt; (D) cobalt. The F-actin microfilaments lost their uniform distribution and aggregated into homogeneous masses after exposure to tungsten carbide/cobalt (C) and cobalt (D). F-actin changes were less significant after exposure to tungsten (B). Bar is 5 μm.

FIG. 2. Phase contrast micrographs of rat lung epithelial cells exposed to 1 μg/ml of hard metal particles: (A) control; (B) tungsten; (C) tungsten carbide/cobalt; (D) cobalt. Phase contrast microscopy revealed little change in cell morphology. Bar is 50 μm.

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