INTRODUCTION
The Moon’s surface is covered by a layer of reactive dust, containing 1-2% of respirable fine dust (< 3 µm). The habitable area of any lunar landing vehicle would inevitably be contaminated with lunar dust that could pose a health risk. The purpose of the study is to evaluate the toxicity of Apollo moon dust in rodents through inhalation to assess the health risk of dust exposures to humans and to identify the mechanisms and potential pathways involved in lunar dust-induced toxicity.

MATERIAL AND METHODS

1. Animals and dust exposure: Pathogen-free Fischer 344 adult male rats (150-250 g; ~8-10 weeks old at arrival) were purchased from Charles River and were allowed to acclimate for 1 week before they were used in experiments. In each experiment, 132 male Fischer rats were randomly divided into different dose groups (control air, 2.1, 6.8, 21, or 61 mg/m³) and were placed in Battelle rat restraint tubes, which were then connected to the 24 nose-ports of a Jaeger-NYU nose-only chamber. All animals were exposed 6 hours daily, 5 days a week for a total of 120 hours. After each daily 6-hour exposure, the animals were returned to the vivarium, housed in pairs, and observed for clinical signs.

2. Collection and assessment of bronchoalveolar lavage (BAL) cells: At day 1, day 7, 4 weeks, and 13 week post the last exposure, five animal per dose group were sacrificed. The bronchoalveolar lavage fluid (BALF) was collected by lavaging the right lung lobes with a total of 24 ml of phosphate-buffered saline (PBS) in five times. The lavage was centrifuged, and the cell pellets were suspended in 1 ml of HEPES-buffered solution (pH 7.4) containing 1% fetal bovine serum (FBS), 0.05% sodium azide, 100 U/ml penicillin, 100 µg/ml streptomycin, and 250 µg/ml amphotericin B. The BALF was centrifuged, and the cell pellets were suspended in 1 ml of HEPES-buffered solution (pH 7.4) containing 1% fetal bovine serum (FBS), 0.05% sodium azide, 100 U/ml penicillin, 100 µg/ml streptomycin, and 250 µg/ml amphotericin B. The BALF was then processed to determine the cell viability by the trypan blue exclusion method.

3. Tissue collection and gene expression analysis: The lavaged lung tissue was snap frozen in LN2. The expressions of 84 fibrosis related genes were analyzed using the RT2 Profiler PCR Array technique. After DNase treatment, 1 µg total RNA of each of the three samples per time-point and per group animals that were exposed to control air or high dose lunar dust particles (60 mg/m³) was used to synthesize cDNA using a RT2 PCR array first strand kit. Real-time PCR Analysis on 18 Genes of Interest (Table 1).

RESULTS

1. Expression changes in fibrosis related genes after exposure to 61 mg/m³ lunar dust particles:

Figure 1 shows the fold changes of gene expressions in the lung tissue of the animals exposed to 61 mg/m³ lunar dust in comparison to the controls at 13 weeks post-exposure.

Table 1: List of genes that were significantly up- or down-regulated in the lung tissue after dust exposure. (p<0.05, n=3 per control or 61mg/m³ dust exposed group; ↑: Fold change between 1.5 to 2.0; ↓↓: Fold change > 2). Genes of interest were highlighted.

CONCLUSION

- Ccl3, Ccl12, Cxcl2, and Cxcl5 showed persistently significant expression changes in the lung tissue.
- The expression of several of these genes were dose- and time-dependent, and were significantly correlated with other pathological.
- Our previous data showed that no pathological changes were detected in low dose groups. However, several genes, primarily produced by lung epithelial, were significantly altered persistently in response to low-dose dust exposure.
- The data presented in this study, for the first time, explores the molecular mechanisms of lunar dust induced damage in response to lunar dust exposure in vivo. It also provides insight into the potential risk assessment for future space exploration, but also understandings of the dust-induced toxicity to humans on earth.

ACKNOWLEDGEMENT

This work was supported by the NASA Space Radiation Health Program.