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Original Article

Research on rat's pulmonary acute injury induced by lunar soil simulant

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Abstract

Background: The steps to the moon never stopped after the Apollo Project. Lessons from manned landings on the moon have shown that lunar dust has great influence on the health of astronauts. In this paper, comparative studies between the lunar soil simulant (LSS) and PM2.5 were performed to discover their harm to human biological systems and explore the methods of prevention and treatment of dust poisoning for future lunar manned landings.
Methods: Rats were randomly divided into the control group, two CAS-1 lunar soil simulant groups (tracheal perfusion with 7 mg and 0.7 mg, respectively, in a 1-mL volume) and the PM2.5 group (tracheal perfusion with 0.7 mg in a 1-mL volume). The biochemical indicators in the bronchoalveolar lavage fluid (BALF), MPO activity in the lung tissue, pathologic changes, and inflammatory cells in the BALF were measured after 4 h and 24 h.
Results: The LSS group showed cytotoxicity that was closely related to the concentration. The figures of the two LSS groups (4 and 24 h) show that the alveolar septa were thickened. Additionally, it was observed that neutrophils had infiltrated, and various levels of inflammation occurred around the vascular and bronchial structures.
Conclusion: The overall results of the acute effects of the lungs caused by dust showed that the lung toxicity of LSS was greater than that of PM2.5. LSS could induce lung damage and inflammatory lesions. The biomarkers in BALF caused by acute injury were consistent with histopathologic observations.

Keywords: Acute injury; Lunar soil simulant; Lung; Rat

1. Introduction

Lunar exploration projects in China have steadily implemented with the successes of the ChangE 1, 2, and 3. The ChangE 4, with the aim to land on the far side of the moon, and ChangE 5, with the aim to collect lunar samples to be analyzed on Earth, have already been planned to launch in the coming years. Thereafter, China also has the strategy to send astronauts to the lunar surface.[1] However, lunar dust is the primary environmental challenge that astronauts must face during manned landings. How to protect the health and safety of astronauts exposed to lunar dust is one of the most important issues to research.

Lunar dust comprises common tiny particles on the lunar surface. The diameter of lunar soil particles is between 40 μm and 800 μm, with the average value approximately 70 μm.[2] Among them, lunar dust with a diameter lower than 20 μm is researched in this paper, accounting for 20% of all lunar

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dust at a depth of 1−2 cm of the lunar surface, and they are primarily composed of very fine particles.[3,4] The chemical components of lunar dust includes approximately 50% SiO_2, 15% Al_2O_3, 10% CaO, 10% MgO, 5% TiO_2, 5%−15% Fe, and small amounts of Na, K, Cr and Zr; among them, np-Fe is considered the main factor leading to the toxicity of lunar dust, and it mainly exists at the edge of the dust particles.[5] Currently, whether np-Fe directly contacts the body remains unclear. Because it takes a long time to clear lunar dust particles, np-Fe is likely to remain in contact with the cells and tissues directly in vivo for an extended period.

After the Apollo lunar missions in the 1960s, US President Bush appealed to NASA to treat lunar dust as a stepping stone to Mars in 2004. It was predicted that human beings will be exposed to lunar dust for extended periods of time in future lunar bases; thus, it is critical to confirm the toxicity of lunar dust before lunar manned landings. The largest hazard of lunar dust is inhalable particles. Lessons from the Apollo Project showed that a flight surgeon who inhaled lunar dust during the unpacking of spacesuits from stowage experienced respiratory immunological symptoms, which progressively worsened after exposure following two subsequent missions.[6]

Based on the experiences and data from the Apollo Project, NASA scientists implemented a series of studies to evaluate lunar dust exposure, but the results could not clearly explain whether the dust caused inhalation injury.[7] Russia has also studied lunar dust, showing that the dust can cause animal lung fibrosis and other symptoms of pneumonia.[8,9] Although the above studies have provided useful explanation about lunar dust toxicity, especially pulmonary toxicity, there is still a lack of experimental evidence, especially comparative studies on the biological toxicity between atmospheric PM2.5 particles from Earth and lunar dust. This study not only intuitively presented evidence of the lunar dust toxicity from the pathological perspective using immune cell counting and classification results but also further discussed the indicators associated with inflammation. These three detection results were interrelated, showed mutual support and are the most comprehensive experimental studies on acute toxicity of lunar dust in current research.

Real lunar dust samples are rare on earth. Even in the United States, a real lunar dust sample is very precious, and usually a lunar soil simulant is used in all types of scientific research and experiment. In this paper, we used the lunar dust analog CAS-1, a Chinese standard LSS, which has a similar chemical and mineral composition to lunar dust, as an alternative to explore lunar dust toxicity. The lunar soil simulant was confirmed to be CAS-1 simulated lunar soil with similar material composition and physicochemical properties to the lunar soil associated with the Apollo 14 landing.[10]

2. Methods

2.1. CAS-1 lunar soil simulant and PM2.5 air particles

Most of the lunar soil analogs are materials from Earth that have similar mineral composition, chemical composition, physical properties, and mechanical properties to real lunar soil. The ejecta from the Jinlongdingzi volcano within the Longgang volcanic swarm located in the Jilin province of north China are used as the raw material to prepare CAS-1 lunar soil, a Chinese standard LSS. A detailed description and comparison have been provided previously.[10] The CAS-1 lunar soil simulant and lunar sample from Apollo 14 have almost the similar mineral composition, chemical compositions, and physical and mechanical properties. The CAS-1 lunar soil analog contains 20%−40% of glass material and is an ideal low-Ti basaltic lunar analog. Lunar soil analogs are obtained by corundum roller milling. In the process of grinding, metal contamination should be avoided. After measuring multiple times, the average diameter of the lunar soil simulant is 85.938 μm, which is very close to the average size of the lunar samples from the Apollo 14 landing sites. To acquire a suitable size of CAS-1 simulated lunar dust for inhalation, we use 5-μm aperture sieving to screen samples. Thus, the CAS-1 simulated lunar dust with a diameter size under 5 μm could be collected.

PM2.5 suspended particles were collected using a TE-6070 high volume air sampler (Tisch Environmental Inc, Miami, FL, USA). The composition analyses of the samples were described by Ma et al.,[11] who showed that they contained a large amount of heavy metals such as Mn, Zr, Cu, Cr and Ca and polycyclic aromatic hydrocarbon substances such as anthracene, chrysene and benzo(a)pyrene.

The samplers worked in the industrial and traffic arteries 24 h a day and consecutively sampled for four months with the average sampling flow of 6−8 L/min. Next, we cut the sampled filter paper into a 1 × 1 cm size and immersed it in distilled water with ultrasonic shaking for 20 min with a multilayer gauze filter for filtration by centrifugation at 1000 rpm at 4 °C. Finally, the pellet was collected, vacuum frozen, dried, and stored at −80 °C.

2.2. Animal grouping

During the experiments, 48 SPF male Wistar rats weighing 190 g−210 g were chosen from the Liaoning Changsheng Biotechnology Co. Ltd (Animals License number: SCXK (Liao) 2010-0001). They were fed and housed in polycarbonate cages (equipped with HEPA air filter) for one week, and their daily activities and eating were observed. After being weighed, they were equally divided into 8 groups: 4-h and 24-h control saline groups ($n = 6$ per group), 4-h and 24-h PM2.5 groups ($n = 6$ per group), low-dose 4-h and 24-h LSS groups ($n = 6$ per group), and high-dose 4-h and 24-h LSS groups ($n = 6$ per group).

2.3. Intratracheal instillation of dust samples

The intratracheal instillation method was used for the administration of the dust samples. First, the rats were fixed onto a framed platform and restrained after being anesthetized with ether. Next, we used swabs to remove secretions from the throat of the rats. Second, under a transmissive lamp, we adjusted the tilt angle of the rats to clearly observe the rima glottidis through their oral cavity. Next, we placed the endotracheal tubes through the rima glottis of the rats and

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