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RESEARCH ARTICLE

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Exploring the mechanism of lung injury induced by lunar dust simulant in rats based on metabolomic analysis

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Abstract

Inflammatory response and oxidative stress are considered to be important mechanisms of lung injury induced by lunar dust. However, the pulmonary toxicological mechanism remains unclear. In the present study, Wistar rats were exposed to CLDS-i 7 days/week, 4 h/day, for 4 weeks in the mouth and nose. Lung tissue samples were collected for histopathological analysis and ultra-performance liquid chromatography–mass spectrometry analysis. Enzyme activities and expression levels of key metabolic enzymes were detected by biochemical analysis and real-time PCR. The pathological features of lung tissue showed that CLDS-i caused congestion and inflammation in the lungs, and the lung structure was severely damaged. Metabolomics analysis showed that 141 metabolites were significantly changed in the lung tissue of the CLDS-i group compared with the control group. Combined with Kegg pathway analysis, it was found that the changes of amino acid metabolites were involved in these pathways, indicating that the simulated lunar dust exposure had the most obvious effect on amino acid metabolism in the lung tissue of rats. Real-time PCR analysis showed that the mRNA expression of six key enzymes related to amino acid metabolism was changed, and the enzyme activities of these key enzymes were also changed, which were consistent with the results of qPCR. These results suggest that changes in amino acid metabolism may be closely related to the pathogenesis of lung injury induced by lunar dust, and amino acid metabolism may be a potential biomarker of lung diseases related to lunar dust exposure.

KEYWORDS
amino acid metabolism, differential metabolites, lunar dust simulant, metabolomics, pulmonary toxicity

1 | INTRODUCTION

Lunar dust particles are the tiny particles covered on the lunar surface. Due to the charged nature of the lunar dust particles and the effect of the electric field around them. They easily suspend in the air. When the astronauts probe the lunar surface, these dust particles will stick to the helmets, space suits, and boots, making the astronauts' vision unclear and inconvenient to move. In addition, such particles with irregular shape and extremely complex structure will also cause wear and tear on space suits and detection equipment, posing a huge challenge to human landing on the moon. Studies have shown that exposure to lunar dust can cause a variety of adverse health effects, such as chronic bronchitis, chronic fibrosis of lung tissue, cardiovascular disease, skin inflammation, and conjunctivitis.

Due to the scarcity of real lunar dust samples, simulated lunar dust samples are currently used to study the toxicity of lunar dust

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particles. Studies have shown that exposure of lunar dust simulated samples to the lungs caused local and systemic inflammatory responses in rats, significantly increased parasympathetic nerve activity, aggravated myocardial fibrosis, and ultimately led to myocardial dysfunction and cardiovascular damage.[1] The mechanism of cardiotoxic damage involves inflammatory mechanism, considering that NOX4 may be a potential therapeutic target of lunar dust simulant (LDS) induced myocardial injury.[1] Lam et al. studied the pulmonary acute toxicological effects of lunar dust simulants in mice and found that lunar dust was more toxic than titanium dioxide, but less toxic than silica-containing quartz dust.[2] Compared with the low-dose lunar dust group, the lung tissue of mice in the high-dose lunar dust simulant group showed mild to moderate alveolitis, as well as perivascular and peribronchiolar inflammation; after 90 days of exposure, the lung tissue showed mild Chronic lung inflammation, septal thickening, and some signs of fibrosis.[3] Stained sections of rat lung tissue exposed to simulated lunar dust showed different degrees of inflammatory lesions and fibrosis under microscope.[4] These results suggest that with prolonged exposure to simulated lunar dust and increasing doses, acute inflammatory responses in animals' lungs can be transformed into chronic inflammatory lesions in a dose-time-dependent manner. Sun et al. exposed rats to two doses of lunar dust simulant by tracheal instillation, and measured related immune and oxidative indicators in bronchoalveolar lavage fluid (BALF) of rats. The results confirmed that under the effect of simulated lunar dust, inflammation, oxidative stress and immune response promote each other, causing pulmonary vascular endothelial cell damage and lung inflammation, and inducing lung injury.[4–6] However, the pathogenesis of lung injury induced by simulated lunar dust has not been clearly elucidated. Therefore, more in-depth studies are needed to better understand the pathogenic process of lunar dust simulants exposure leading to pulmonary toxicity.

Metabolomics reveals the dynamic changes of various metabolites caused by the pathological state of the living system by analyzing the changes of metabolites after the biological system is stimulated by external factors, describing the phenotype of the organism and studying the pathological mechanism of the occurrence and development of diseases.[7] In the PM2.5 exposure study, some scientists found that the lung metabolome of rats exposed to PM2.5 for a long time changed significantly. Combined with the results of oxidative stress, PM2.5 can induce pulmonary toxicity by disturbing the pro-oxidative/anti-oxidative balance.[8] Metabolomics analysis showed that its differential metabolites were mainly involved in the citric acid cycle, glyoxylate and dicarboxylic acid metabolism, pyruvate metabolism, purine and pyrimidine metabolism, and valine, leucine and isoleucine metabolism and other pathways.[7] When nuclear magnetic resonance (NMR)-based metabolic methods were used to study the changes in the metabolic components of A549 cells treated with PM2.5, 12 metabolites were significantly changed, and the related metabolic pathways involved are similar to the results of Li et al., mainly involving amino acid metabolism, lipid metabolism, nucleotide metabolism, and the tricarboxylic acid cycle.[9] In addition, in experiments with pregnant mice, the mRNA levels of the inflammatory mediator Pla2g2d and its metabolites lysophosphatidylcholines (LysoPCs) and arachidonic acid (AA) were up-regulated in the lung tissue of PM2.5 group, by contrast, these inflammatory changes were recovered after treatment with mogrosides (MGs) during pregnancy; Pla2g2d was suggested to be a potential target of MGs to ameliorate PM2.5-induced lung injury.[10] These studies all confirm that metabolomic approaches are effective tools to characterize metabolic changes in air pollution-induced lung injury.[11] Lunar dust, as the fine dust particles existing on the lunar surface, has a lung toxicity far greater than that of PM2.5 due to its special physical and chemical properties. In the past, studies on lung injury induced by lunar dust simulant did not focus on the metabolomics study of lung tissue, and the relationship between lunar dust simulant exposure and metabolites in lung tissue was unclear. Therefore, this study was based on metabolomic analysis of metabolite changes in the lung tissue of simulated lunar dust-exposed rats to search for potential biomarkers to reveal its underlying pathogenesis.

2 | MATERIALS AND METHODS

2.1 | Preparation of simulated lunar dust suspension

The simulated lunar dust sample CLDS-i used in this study was developed by the Institute of Geochemistry, Chinese Academy of Sciences. The researchers further processed the simulated lunar dust sample CLRS-1 developed earlier, and obtained CLDS-i, which is very similar to the real lunar dust in terms of mineral composition, physicochemical properties, grain size and nano-metal iron properties. Most of the particles in CLDS-i are less than 1 μm in diameter.[6] After autoclaving, vacuum freeze-drying, and store at −80°C. Preparing simulated lunar dust suspension with normal saline for use.

2.2 | Animal protocol

Twenty-eight healthy SPF grade male Wistar rats, weighing 190–220 g were obtained from Beijing Huafukang Biotechnology Co. Ltd. (Animals License number: SCXK [Jing] 2019–0008). They were placed in polycarbonate cages (equipped with HEPA air filters), 2 animals/cage, 12 h-12 h light/dark cycle, room temperature: 24°C ± 2°C, relative humidity: 50% ± 5%, with free access to food and water, and were reared for 1 week to observe their activities and feeding. Then they were weighed and randomly divided into two groups: simulated lunar dust group and normal saline control group, with 14 animals in each group.

The Wistar rats in the experimental group were placed in the oral and nasal exposure chamber (Beijing Huironghe Technology Co., Ltd.) to be exposed to dust at a concentration of 7 mg/m^3 CLDS-i for 4 h/d, 7 d/week for 4 weeks, and the control group was exposed to normal saline under the same conditions. In accordance with the requirements of inhalation toxicity Testing guidelines issued by the Organization for Economic Co-operation and Development (OECD), The system Settings are as follows: aerosol flow rate: 2 L/min, dilution

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