

EFFECTS OF LOW TO MEDIUM DOSE PROTON IRRADIATION ON GUT MICROBIOTA IN BALB/C MICE

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Abstract

Proton radiation dominates space radiation (~ 90%) and threatens deep space missions, yet its effects on gut microbiota at mission-relevant doses (0.1-1 Gy) are unclear. This study assessed impacts in BALB/c mice exposed to whole-body proton irradiation (0, 0.1, or 1 Gy; 100 MeV cyclotron). Fecal samples (day 3 post-irradiation) underwent 16S rRNA sequencing. Results revealed a dose threshold: 1 Gy induced significant structural remodeling, marked by increased Firmicutes (56.93% vs. control 32.23%), decreased Patescibacteria (3.95% vs. 27.95%), a 116% higher Firmicutes/Bacteroidetes ratio, and enrichment of biomarkers *Lachnospiraceae_NK4A136_group* (LDA=4.2) and *Deferribacteres* (LDA=3.8). Conversely, 0.1 Gy caused minor changes (*Rikenellaceae*, LDA=3.5; *Odoribacter*, LDA=3.3). Critically, 1 Gy enhanced alpha diversity (Chao1 +15-20%, Shannon +11.3%; $p < 0.05$) and altered beta diversity ($R^2 = 0.251-0.361$, $p < 0.05$) versus controls. Functional prediction (PICRUST2) identified disruptions in cellular processes/signaling ($p = 0.0133$), poorly characterized pathways ($p = 0.0298$), ABC transporters (K03088, $p = 0.021$), and fatty acid metabolism (K02003, $p = 0.045$). These findings demonstrate that gut microbiota exhibits differential dose-response patterns to proton radiation, providing both reference biomarkers and mechanistic insights for evaluating astronaut intestinal health risks and developing microbiota-targeted countermeasures during deep space missions.

INTRODUCTION

With human space exploration progressively extending from low-Earth orbit to deep space, long-duration missions such as crewed lunar landings and Mars exploration have become strategic goals for major

spacefaring nations [1]. However, space radiation poses one of the most severe challenges to manned missions in the deep space environment[2]. Space radiation has a complex composition, with proton radiation accounting for approximately 90% of its constituents, making it the primary source of radiation exposure for astronauts . Unlike the space station environment, which is protected by Earth's magnetic field, spacecraft on deep space missions face significantly higher radiation intensities. It is estimated that astronauts may receive a cumulative radiation dose as high as 660 mSv or more during a round-trip to Mars[2]. This persistent radiation exposure can not only directly cause DNA damage and increase the risk of cancer, but may also lead to long-term harm to the central nervous system, cardiovascular system, and other organs[3,4].

Among radiation-sensitive organs, the intestine is a critical target for radiation damage due to the high turnover rate of its epithelial cells[5-7]. Radiation can disrupt the normal proliferation and differentiation of intestinal epithelial cells, compromise the integrity of the intestinal barrier, and lead to the translocation of microbes and endotoxins, thereby triggering a systemic inflammatory response[8,9]. In recent years, the role of the gut microbiota, often hailed as the host's 'second genome', in the radiological response has garnered increasing attention[10,11]. Numerous studies have shown that the gut microbiome plays a central role in maintaining intestinal health by modulating host metabolism, immune homeostasis, and mucosal barrier function[12,13,14]. Radiation exposure can significantly disrupt the ecological balance of the gut microbiota (dysbiosis), manifested as altered microbial diversity, a decrease in beneficial bacteria, and the proliferation of potential pathogens[15]. This dysbiosis is not only a biomarker of radiation injury but may also amplify or

mitigate the systemic effects of radiation on the host through bidirectional communication pathways such as the 'gut-brain axis' and 'gut-liver axis'.

Currently, research on the effects of radiation on the gut microbiota has predominantly focused on conventional gamma rays or X-rays. In contrast, studies on proton radiation, especially the effects of low-to-moderate dose (0.1-1 Gy) proton radiation, are relatively scarce. As the main component of space radiation, protons differ from photon radiation in their physical characteristics (e.g., energy deposition pattern characterized by the Bragg peak) and relative biological effectiveness (RBE). A limited number of studies suggest that proton radiation may have selective effects on specific functional groups of bacteria, such as butyrate-producing bacteria, and that its effects are dependent on the host's genetic background. However, for low-to-moderate dose proton radiation, which is more relevant to the actual exposure levels of astronauts, the dose-response relationship, key sensitive microbial biomarkers, and functional alteration patterns remain unclear.

Therefore, this study aims to systematically investigate the effects of whole-body irradiation with low-to-moderate dose (0.1 Gy and 1 Gy) protons on the gut microbiota of BALB/c mice. By establishing a standardized animal irradiation model and combining 16S rRNA high-throughput sequencing with bioinformatic analysis, we will elucidate the dose-dependent effects of proton radiation from three dimensions: microbial community structure, diversity dynamics, and predicted functional potential. The findings are expected to provide experimental evidence for clarifying the mechanisms of intestinal microecological disruption in the space radiation environment and offer important theoretical support for the development of biomarkers for astronaut radiation damage and microecological protection strategies.

EXPERIMENTAL DETAILS

In this experiment, we used SPF-grade female BALB/c mice (n=18), which were divided into three groups: 0 Gy, 0.1 Gy, and 1 Gy. Whole-body irradiation was conducted on the 100 MeV high current proton cyclotron at the China Institute of Atomic Energy, with a dose rate of 0.1 Gy/min. Fecal samples were collected on

the third day after irradiation, and DNA was extracted. The 16S rRNA V3-V4 region was amplified and sequenced using the Illumina platform with PE250. OTU clustering, species annotation, diversity analysis, and functional prediction were performed using QIIME2 and usearch software. PICRUSt2 was used for common and unique species statistics, community composition analysis, and species abundance clustering analysis (using R software). LEfse software was used for significant difference analysis of species between groups, and linear discriminant analysis (LDA) was used for dimensionality reduction and assessment of the impact size of significantly different species.



Figure 1: the CYCIAE-100 high current proton Cyclotron

RESULTS AND DISCUSSION

This research systematically explored the impacts of medium- and low-dose proton irradiation on the intestinal microbiota of BALB/c mice via 16S rRNA high-throughput sequencing. The findings demonstrated that medium-dose irradiation at 1 Gy exerted a highly notable influence on the structure of the intestinal microbiota. At the phylum level, 1 Gy of irradiation caused the relative abundance of Firmicutes to increase remarkably from 32.23% in the control group to 56.93%. Conversely, the relative abundance of Patenscibacteria decreased from 27.95% to 3.95%. Moreover, the ratio of Firmicutes to Bacteroidetes (F/B) soared by 116% (Figure 2.a). At the genus level, the alterations were even more pronounced. The abundance of the Lachnospiraceae_NK4A136_group, which is closely associated with the production of short-chain fatty acids (SCFAs), increased significantly from 7.50% to 31.93%. In contrast, the abundance of Candidatus_Saccharimonas plummeted from 30.51% to 4.43%. In comparison,

