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Protective effects of ginsenosides on macrophages subjected to simulated weightlessness

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ABSTRACT

Objective To investigate the evolution of inflammation under conditions and the effects of ginsenosides on macrophages subjected to the simulated weightlessness, with the aim of mitigating the inflammation.

Methods Initially, genes related to weightlessness, inflammation, and immunity were identified in the GeneCards database. Then, Search Tool for the Retrieval of Interaction Gene/Proteins (STRING) protein network analysis was conducted to determine the core targets involved in the weightlessness-induced inflammation. Subsequently, Label-Free Quantitative (LFQ) proteomics was carried out to discern the distinctive genes within ginsenoside-treated Tohoku Hospital Pediatrics-1 (THP-1) cells. Next, utilizing the outcomes of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, the biological processes and signaling pathways in which ginsenosides predominately engaged were scrutinized, and the primary targets of ginsenosides in combating weightlessness-induced inflammation were examined. Finally, enzyme-linked immunosorbent assay (ELISA) was performed to detect the secretion levels of interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α from lipopolysaccharide (LPS)-induced THP-1 cells under simulated weightlessness conditions, as well as during the weightlessness recovery period following treatment with ginsenosides.

Results A total of 2 933 genes associated with inflammation, 425 genes linked to weightlessness, and 4 564 genes connected to immunity were retrieved from the GeneCards database. Protein-protein interaction (PPI) networks were generated to identify pivotal targets associated with weightlessness-induced inflammation such as IL-1β, IL-6, TNF, and albumin (ALB). It was found that ginsenosides primarily participated in the regulation of various inflammation-related signaling pathways and pathways related to pathogenic microorganism infections. Moreover, it has a significant impact on the expression of proteins such as cluster of differentiation 40 (CD40), IL-1β, and poly ADP-ribose polymerase 1 (PARP1). As revealed in the simulated weightlessness cell test, ginsenosides exhibited a remarkable capacity to attenuate the secretion of inflammatory factors, specifically IL-6 and TNF-α ($P < 0.000\ 1$), in THP-1 macrophages following induction by LPS under simulated weightlessness conditions. In addition, it reduced the secretion of IL-1β, IL-6, IL-8, and TNF-α ($P < 0.000\ 1$) during the weightlessness recovery phase.

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Conclusion Weightlessness can disrupt several inflammation-related signaling pathways, but ginsenosides were shown to mitigate the release of various inflammatory factors in macrophages subjected to simulated weightlessness, thereby exerting a protective role against inflammation. This study has laid a theoretical groundwork for further exploring the potential application of ginsenosides in safeguarding against LPS induced inflammation in a weightlessness environment.

1 Introduction

Astronauts are susceptible to a range of physiological changes, including accelerated degradation of muscular and skeletal tissues [1, 2], dysbacteriosis [3], cardiovascular disease [4], and perturbations to the immune system [5-7]. These changes can be attributed to factors such as solar radiation, disruptions in circadian rhythms, stress, and microgravity. In the aforementioned systems, immune system injury persists during spaceflight, elevating risks of astronaut infections both during and after space missions [8]. During the Apollo program, a notable number of astronauts contracted bacterial or viral diseases, such as conjunctivitis, upper respiratory tract infections, and gastrointestinal discomfort [9]. Simultaneously, clinical data obtained from the 46 crew members aboard the International Space Station indicated that rhinitis, rashes, and upper respiratory infections were the prevailing diseases observed among them [10]. In addition, CRUCIAN et al. [7] observed alterations in leukocyte distribution, T cell activity, and cytokine production during the space flight. Having utilized a microgravity zebrafish model, ZHU et al. [11] discovered that in the microgravity environment, signaling pathways associated with the retinoic acid-inducible gene I- like receptor (RLR) and the toll-like receptor (TLR) were markedly suppressed, resulting in a diminished antiviral function. During a 22-day space-flight carrying mice, researchers observed the impairment of the thymus and lymphatic glands, coupled with a decline in lymphocyte counts [12]. Similarly, according to the findings by BAQAI et al. [13], mice exposed to a 13-day spaceflight exhibited reduced spleen and thymus gland sizes, alongside heightened production of interleukin (IL)-6 and IL-10 by lymphocytes. In simulated weightlessness cell assays, it has been noted that microgravity can lead to a decrease in the number of intestinal epithelial cells [14]. Through weightlessness simulation, JIANG et al. [15] unveiled that microgravity induced endoplasmic reticulum (ER) stress in human umbilical vein endothelial cells (HUVEC) cells, leading to the activation of nuclear factor kappa-B/inhibitor of NF-κB (NF-κB/IκB) and nucleotide oligomerization domain (NOD)-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome signaling pathways. These pathways played an essential role in inducing endothelial inflammation and apoptosis. In other words, a microgravity environment could potentially harm the immune system by modulating the release of inflammatory factors and altering the number of lymphocytes. Hence, understanding the impacts of microgravity on immune cells and devising appropriate preventive measures are essential prerequisites for protecting the health of astronauts.

As one of the main components identified in Renshen (Ginseng Radix et Rhizoma), ginsenosides show a variety of pharmacological properties that have been investigated for their potential therapeutic applications in addressing conditions such as ageing, cancer, cardiovascular disease, and diabetes [16-20]. Drawing from a type 1 diabetes mouse model [21], it was demonstrated that ginsenosides Rg1 held promise for mitigating the inflammatory response and autophagy processes in both the pancreas and spleen. LUO et al. [22] reported that ginsenosides Rg1 exhibited the potential to diminish cardiomyopathy and inflammation through the regulation of the TLR4/NF-κB and NLRP3 pathways. In a research on chronic inflammatory mice model, LIU et al. [23] discovered that ginsenosides Rg3 inhibited the autophagy signaling pathway mediated by inflammations, thereby retarding the progression of liver fibrosis. Furthermore, the therapeutic effects of ginsenosides extended to the reduction of intestinal inflammation through inhibiting the TLR4-myeloid differentiation factor 88 (MYD88)-mitogen-activated protein kinase (MAPK) signaling pathway [24]. Additionally, ginsenosides revealed the ability to regulate the Treg/Th17 cell ratio and mitigate inflammation, suggesting their potential as a treatment option for chronic obstructive pulmonary disease [25]. Therefore, ginsenosides hold promise for application in protecting against inflammation and immune function injury.

Because of the substantial expenses associated with performing weightlessness research during actual spaceflight, it becomes imperative to employ simulated weightlessness techniques in order to investigate its physiological impacts. Therefore, the simulation process was devised and carried out using a cell rotor, which rotated cells uniformly around the horizontal axis to create microgravity conditions. This study initially identified weightlessness-related genes from the GeneCards database, and then investigated weightlessness-related signaling pathways and primary targets with the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. Next, the

mechanism of ginsenosides on Tohoku Hospital Pediatrics-1 (THP-1) cells was explored by Label-Free Quantitative (LFQ) proteomics. Following this, THP-1 cells were cultured on the cell rotor to assess the impact of ginsenosides on their viability and morphology. Finally, enzyme-linked immunosorbent assay (ELISA) was performed to measure the release of IL-1β, IL-6, IL-8, and TNF-α during both the simulated weightlessness period and the subsequent recovery phase. The findings in the study offer a valuable theoretical foundation for further exploring the potential application of ginsenosides in the prevention of weightless-induced inflammation.

2 Materials and methods

2.1 Data sources

Genes related to inflammation, immunity, and weightlessness were retrieved from the GeneCards database (www.genecards.org). To pinpoint common genes across the three sets, a Venn diagram was drawn for the visualization of the intersected genes (bioinfogp.cnb.csic.es/tools/venny).

2.2 Cell culture

THP-1 cells were obtained from the Chinese National Infrastructure of Cell Line Resources (NICR, China) and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and penicillin-streptomycin (Gibco, USA).

2.3 Protein extraction and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

THP-1 cells were induced to differentiate into macrophages through the treatment with 100 ng/mL poly methyl acrylate (PMA) (Sigma, USA) for 72 h. Subsequently, cells in the lipopolysaccharide (LPS) (Sigma, USA) group were exposed to LPS at a concentration of 1 μg/mL and cultured at 37 °C, 5% CO_2 for 48 h. In the LPS + ginsenosides group, cells were subjected to a combined treatment of LPS at a concentration of 1 μg/mL and 100 μg/mL total ginsenosides (provided by The Institute of Medicinal Plant Development). THP-1 cells were lysed on ice for 15 min in 100 μL of Radio Immunoprecipitation Assay (RIPA) lysis buffer (Solarbio, China) containing a protease inhibitor cocktail (Promega, USA). The protein concentration in the lysates was quantified using a Broadcast Credit Association (BCA) protein assay kit (Solarbio, China). Equal amounts of cellular proteins were then suspended in NuPAGE LDS sample buffer (Invitrogen, USA) and NuPAGE™ sample reducing buffer (Invitrogen, USA) before being subjected to boiling for 5 min at 95 °C. Following this, proteins were separated using 4% - 12% Mini-PROTEAN TGX gels (Bio-Rad, USA).

2.4 In gel digestion and proteomic analysis

For Coomassie staining, the bands of interest were cut into 1 mm × 1 mm pieces and subjected to destaining in a destaining solution. Then, the gel pieces were treated with dithiothreitol (DTT) (Sigma, USA) and iodoacetamide (IAM) (Sigma, USA). Lastly, the samples were enzymatically digested overnight at room temperature in a trypsin solution (Promega, USA), and the resulting peptides were stored at – 80 °C before analysis.

Peptide samples were analyzed with the use of the Thermo Orbitrap QE Plus system. The data were collected using Xcalibur 3.0 software, and proteomics data were analyzed using the Proteome Discovery application (version 2.4). To determine the FoldChange, *t* test was conducted on two sets of data using R Studio. The GO and KEGG enrichment analyses were performed using the R package Cluster Profiler.

2.5 Simulated weightlessness cellular model

During the simulated weightlessness period, the cells were divided into five groups: the non-weightlessness group (NW), control (C-SW), ginsenosides (G-SW), LPS (L-SW), and LPS + ginsenosides (LG-SW) groups. After 72 h of treatment with 100 ng/mL PMA, THP-1 cells differentiated into macrophages [26]. In the ginsenosides group, differentiated cells were treated with 100 μg/mL ginsenosides. In the LPS group, the cells were treated with 1 μg/mL LPS, and in the LPS + ginsenosides group, the cells were subjected to the treatment with 1 μg/mL LPS and 100 μg/mL ginsenosides. Once the cultivation container was filled, any remaining air bubbles were removed, and the container was then placed on a cell rotator [27]. Lastly, the cells were rotated (30 rpm) and cultured at 37 °C for 48 h.

During the weightlessness recovery phase, the cells were divided into the control (C-WR), LPS (L-WR), and LPS + ginsenosides (LG-WR) groups. In order to induce the differentiation of THP-1 cells into macrophages, cells in the groups were exposed to a concentration of 100 ng/mL PMA for 72 h. Subsequently, after filling the cultivation container and ensuring the removal of any air bubbles, the cells were placed on a cell rotator, and were rotated at 30 rpm and cultured at 37 °C for 48 h. Following this culture period, the cells were collected. In the LPS group, the cells were treated with a concentration of 1 μg/mL of LPS. In the LPS + ginsenosides group, different concentrations of total ginsenosides (100, 33, and 11 μg/mL) as well as 1 μg/mL of LPS were employed. The cells were then cultured at a temperature of 37 °C with 5% CO_2 for 6 h.

2.6 Cell viability

Cell viability was assessed using Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies Inc., Japan) and lactate dehydrogenase (LDH) (Nanjing Jiancheng Bioengineering Institute, China).

For the CCK-8 assay, cells were cultured in the 96-well plate (6 000 per well). The medium was carefully aspirated, and then replaced with fresh medium containing a concentration of 10% CCK-8 solution in each well. Next, the plate was incubated at 37 °C for 1 - 4 h, and the absorbance was measured at 450 nm using an EnSpire Multilabel Plate Reader (PerkinElmer, 2300). Cell viability was calculated by optical density (OD) using the formula: cell viability = [(OD value of sample wells – OD value of blank wells)/(OD value of control wells – OD value of blank wells)] × 100%.

Cell viability was also detected by LDH according to the manufacturer's guidelines using the formula: LDH activity (U/L) = [(OD value of sample wells – OD value of control wells) / (OD value of standard wells – OD value of blank wells)] × 0.2 μmol/mL × 1 000.

2.7 ELISA

The supernatant from the cell culture was obtained by centrifuging at 2 000 rpm for 5 min, and the levels of IL-6, TNF-α, IL-8, and IL-1β were quantified using the ELISA kit (Biolegend, USA) according to the manufacturer 's guidelines.

2.8 Statistical analysis

The data were analyzed using GraphPad Prism 8 software (GraphPad Software Inc.) and were presented as means ± standard deviation (SD). Differences among groups were assessed by one-way analysis of variance (ANOVA) and were considered significant at $P < 0.05$.

3 Results

3.1 Primary targets of weightlessness-induced inflammation based on the GeneCards database

A comprehensive search for genes correlated to inflammation, immunity, and weightlessness from the GeneCards database was carried out. As a result, a total of 231 significant targets involved in the weightlessness-induced inflammation were identified by intersecting the three sets of genes: 2 933 related to inflammation, 425 related to weightlessness, and 4 564 related to immunity (Figure 1A). Subsequently, the protein-protein interaction (PPI) network analysis was performed by Search Tool for the Retrieval of Interaction Gene/Proteins (STRING) analysis, as shown in Figure 1B. It was found that the genes such as albumin (ALB), IL-1β, IL-6, and TNF were key targets of weightlessness-induced inflammation.

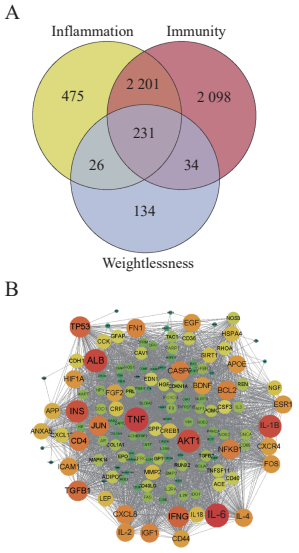

Figure 1 Core genes correlated to weightlessness-reduced inflammation

A, the intersection of genes correlated to inflammation, immunity, and weightlessness. B, PPI network diagram of core genes.

3.2 Protective effects of ginsenosides on THP-1 cells based on the LFQ proteomics

A total of 2 254 proteins were identified by the LFQ techniques, out of which 242 met the criteria as potential targets, with a $\log_2$ FoldChange > 1.5 and $P < 0.05$. Then, the GO and KEGG enrichment analyses were conducted based on these differentially expressed proteins. As shown in Figure 2A, ginsenosides primarily participated in biological processes such as transcription, signal transduction, and innate immune response. Additionally, based on the KEGG analysis results (Figure 2B), ginsenosides predominantly influenced pathways such as the NOD-like receptor signaling pathway, HIF-1 signaling pathway, and multiple pathways related to microbial infections. To further pinpoint the key targets of ginsenosides affecting weightlessness-induced inflammation, the differential proteins from the proteomics analysis were intersected with genes associated with weightlessness and inflammation. As shown in Figure 2C, 10 potential target proteins were identified, including CD40, IL-1β, and poly ADP-ribose polymerase 1 (PARP1), among which IL-1β participated in the regulation of multiple inflammation-related signaling pathways, whose expression also influenced the secretion of a variety of inflammatory factors.

3.3 Effects of simulated weightlessness on cell viability and morphology

To further explore the impacts of ginsenosides on inflammatory factors using a simulated weightlessness cellular model, it was necessary to verify whether simulated weightlessness would affect cell viability. Initially, the

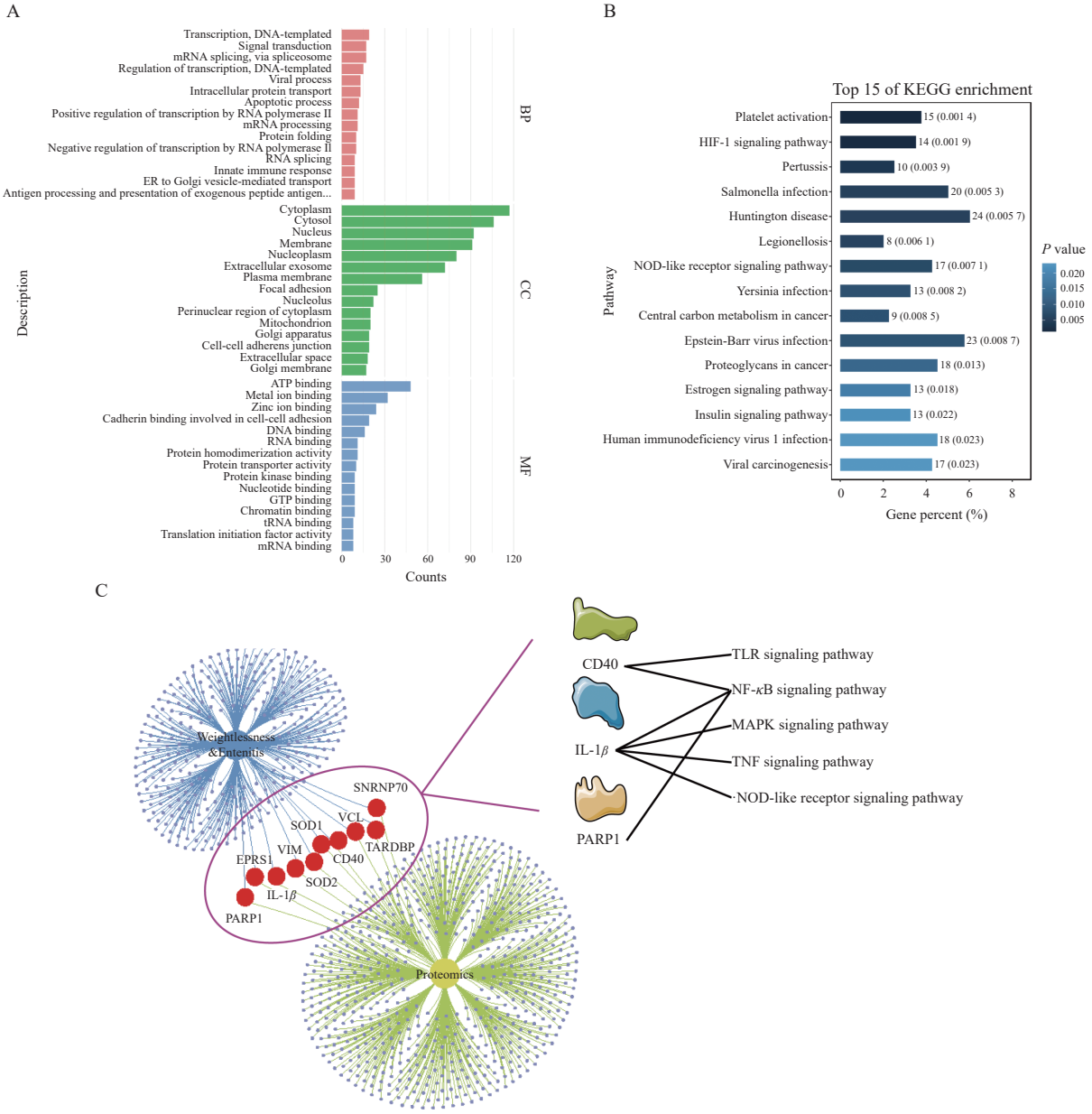

Figure 2 Proteomics analysis of THP-1 cells

A, proteins extracted from cells of both the LPS and LPS + ginsenosides groups. The differential expression proteins, as identified by LFQ proteomics analysis, were subjected to GO enrichment analysis. B, KEGG enrichment analysis. C, a dynamic Venn diagram illustrating the screening process for identifying ginsenosides targeted proteins. BP: biological process. MF: molecular function. CC: cellular component.

cells morphology under a simulated weightlessness condition was observed with the use of fluorescence microscopy in each group. As indicated in Figure 3A, there were no differences found between the NW and the C-SW groups, and between the L-SW and the LG-SW groups. Subsequently, the LDH and CCK-8 assays were performed to detect the cell viability. As illustrated in Figure 3B, there were no significant differences in LDH levels among the C-SW, L-SW, G-SW, and LG-SW groups in THP-1 cells. Furthermore, the result of the CCK-8 analysis also indicated that the presence of ginsenosides at this concentration did not have significant effects on cell viability under simulated weightlessness conditions (Figure 3C). These outcomes provide compelling evidence for the suitability of the model for subsequent experimental investigations.

3.4 Inflammatory factors released during the simulated weightlessness period

To investigate the impacts of ginsenosides on inflammatory factors during the simulated weightlessness period, THP-1 cells were cultured under a simulated weightless environment for 48 h, and the levels of inflammatory factors were assessed using ELISA. As depicted in Figure 4, no expression of inflammatory factors was observed in the C-SW and G-SW groups during the weightlessness

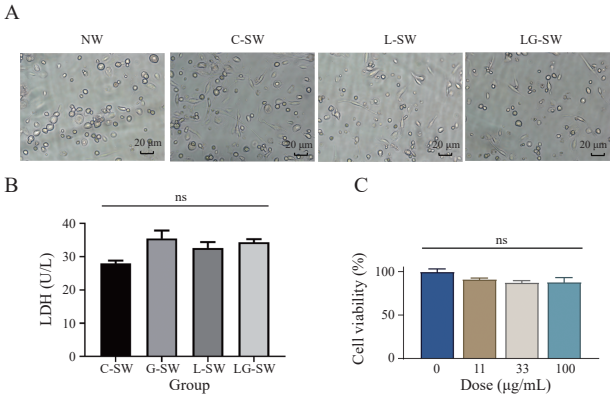

Figure 3 Impacts of weightlessness and ginsenosides on cell morphology and viability

A, fluorescence microscopy. B, LDH assay. C, CCK-8 assay. ns: not significant. NW: non-weightlessness group. C-SW: simulated weightlessness control group. G-SW: simulated weightlessness ginsenosides group. L-SW: simulated weightlessness LPS group. LG-SW: simulated weightlessness LPS + ginsenosides group.

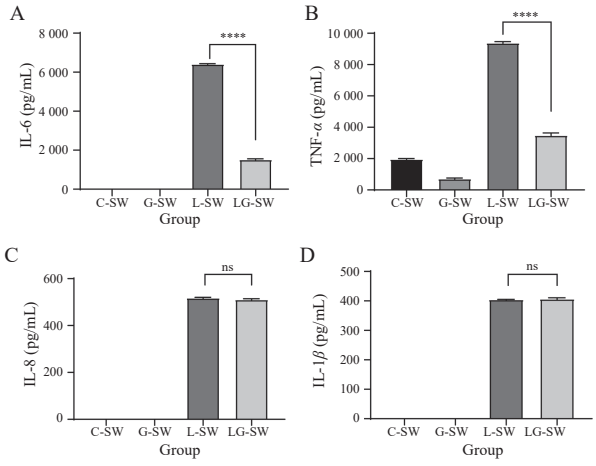

Figure 4 Influences of ginsenosides on cytokines release during the simulated weightless environment

A, IL-6. B, TNF-α. C, IL-8. D, IL-1β. $^{****}P < 0.000\,1$. ns: not significant. C-SW: simulated weightlessness control group. G-SW: simulated weightlessness ginsenosides group. L-SW: simulated weightlessness LPS group. LG-SW: simulated weightlessness LPS + ginsenosides group.

condition. However, after a 48-hour treatment with LPS, there was a notable expression in the levels of IL-6, TNF-α, IL-8, and IL-1β in the supernatant. Notably, the contents of IL-6 and TNF-α in the cell supernatant were significantly reduced after treatment with ginsenosides ($P < 0.000\,1$), while the content of IL-8 and IL-1β remained unchanged. These results suggested that ginsenosides could antagonize LPS-induced inflammatory factors such as IL-6 and TNF-α during the simulated weightless condition.

3.5 Inflammatory factors released during the weightlessness recovery phase

To investigate the impacts of ginsenosides on LPS-induced inflammatory factors during the recovery period, THP-1 cells were cultured for 6 h after the simulated weightlessness period, and the levels of inflammatory factors in the supernatant were detected using ELISA assay. As shown in Figure 5, no inflammatory factors were detected in the control group after the weightlessness recovery phase. However, LPS treatment significantly increased the levels of IL-1β, IL-6, IL-8, and TNF-α in the cell supernatant ($P < 0.000\,1$). Conversely, the treatment with ginsenosides resulted in a substantial reduction of IL-1β, IL-6, IL-8, and TNF-α in supernatant ($P < 0.000\,1$). These results underscored, the ability of ginsenosides to effectively inhibit the release of LPS-induced inflammatory factors, namely IL-1β, IL-6, IL-8, and TNF-α during the weightlessness recovery period.

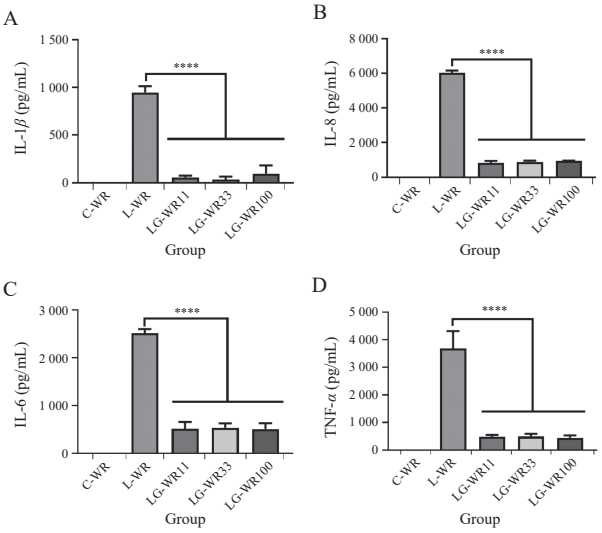

Figure 5 Impacts of ginsenosides on cytokines release during the recovery period

A, IL-1β. B, IL-8. C, IL-6. D, TNF-α. $^{****}P < 0.000\,1$. C-WR: weightlessness recovery control group. L-WR: weightlessness recovery LPS group. LG-WR11/LG-WR33/LG-WR100: weightlessness recovery LPS + ginsenosides group and treated with different concentrations of total ginsenosides (100, 33, and 11 μg/mL).

4 Discussion

As the manned space station program continues to evolve, astronauts will increasingly find themselves spending extended duration in weightless conditions in space. However, prolonged exposure to a weightless environment has detrimental effects on the body's immune system. Astronauts experience alterations in the distribution and function of lymphocytes during spaceflight, leading to changes in the release of various cytokines [7]. A study highlighted numerous instances of virus reactivation during space missions, including viruses such as human herpes virus (HHV), epstein-barr virus (EBV), cytomegalovirus (CMV), and varicella zoster virus [28]. Therefore, it is imperative to explore strategies for protecting immune system in the weightless environment to ensure the health and safety of astronauts.

Research conducted on rhesus monkeys exposed to a prolonged period of microgravity in a weightless environment unveiled the potential for significant alterations in immune function and the occurrence of pulmonary damage. The alteration exerted an influence on the distribution and function of lymphocytes, along with the release of cytokines [29]. To further investigate the primary targets of weightlessness-induced inflammation, this study started with searching genes related to weightlessness, immunity, and inflammation from the GeneCards database, with a total of 231 main intersected genes identified. The STRING database was primarily utilized for protein interaction network analysis, a versatile tool with broad applications in disease research, prediction of core genes for drug development, and network pharmacology [30, 31]. Through the analysis of the STRING interaction networks, it was revealed that IL-1β, IL-6, TNF, insulin (INS), and ALB might be the main targets that were associated with weightlessness-induced inflammation, among which, IL-1β, requiring host-response and pathogen resistance, was an important mediator of the inflammatory response. It also exacerbated damage during chronic illness and acute tissue injury [32]. IL-6 helped host to defense by stimulating acute phase responses, hematopoiesis, and immunological reactions, and the abnormal production of which could result in pathogenesis of chronic inflammation and autoimmunity [33].

More than one study has demonstrated the anti-inflammatory effects of ginsenosides [22, 34]. To determine whether ginsenosides could be used to protect against weightlessness-induced inflammation, the differentially expressed proteins were screened and enriched in the THP-1 cells of the ginsenosides group, and compared with those in the control group using LFQ proteomics. The results indicated that ginsenosides were capable of regulating the infection signaling pathways of various pathogenic microorganisms as well as NOD-like receptor signaling pathways and HIF-1 signaling pathways. Previous studies also found the ability of ginsenosides to mitigate the adhesion of Salmonella, decrease the proportion of Escherichia coli microbiota, and inhibit the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [35-38]. However, the regulation of Yersinia and the human papilloma virus (HPV) infection following ginsenosides treatment have not been reported yet. By intersecting differentially expressed proteins with genes related to weightlessness-induced inflammatory, a total of 10 targeted proteins including CD40, IL-1β, PARP1, and SOD1 were discovered, of which CD40, IL-1β, and PARP1 reported to have regulated the levels of TNF, TLR, NF-κB, and other signaling pathways, leading to changes in the levels of inflammatory factors including IL-6, IL-1β, and TNF-α [39-41]. The results also suggested that ginsenosides might inhibit the release of pro-inflammatory cytokines by modulating the expression of the above target proteins.

To investigate the effects of ginsenosides on inflammatory cytokines in a weightless environment, cell models were constructed under a simulated weightless environment, whose morphology and viability were detected under the specific environment. PMA-induced differentiation of THP-1 cells into M0 macrophages was an ideal model for investigating immune regulation [26]. LPS was the component of the cell wall of gram-negative bacteria, with ability to induce inflammatory responses in host cells and promote M0 macrophages differentiation into M1 macrophages [42]. Compared with cells in the control group, those in the ginsenosides group didn't alter in morphology under the simulated weightless environment. Next, cell viability was determined by employing the LDH and CCK-8 assay, which demonstrated that neither weightlessness nor ginsenosides treatment affected cell viability. M1 macrophages were found to bear the capacity of releasing several inflammatory factors such as IL-1β, IL-6, and TNF-α, thereby contributing to microbial destruction and phagocytosis. In addition, overproduction of inflammatory factors also caused tissue damage, disrupted epithelial barrier, and caused inflammatory response in tissues [43]. Hence, in order to evaluate the protective influences of ginsenosides on cells, this study employed a cell rotator to simulate cellular weightlessness. The THP-1 cells were cultured on the cell rotator and treated with LPS to induce inflammatory responses, followed by the treatment with ginsenosides. ELISA was then utilized to detect the expression of inflammatory factors, and the results demonstrated that ginsenosides could reduce the levels of IL-6 and TNF-α during a simulated weightless environment. Furthermore, ginsenosides also exhibited a noteworthy reduction in IL-1β, IL-6, IL-8, and TNF-α levels in cells during the recovery period after simulated weightlessness. In this study, it was underscored that ginsenosides could mitigate the release of cellular inflammatory factors during simulated weightlessness period and the following recovery phase, suggesting a protective role in combating tissue inflammation during a weightless environment.

5 Conclusion

In summary, IL-1β, IL-6, and TNF were found to be the primary targets of weightlessness-induced inflammation via STRING protein network analysis. Proteomics analysis of THP-1 cells revealed that ginsenosides also primarily participated in a variety of inflammation-related and microbial infections signaling pathways, as well as significantly altered the expressions of inflammation-related proteins such as CD40 and IL-1β. Ginsenosides were found to have the ability to markedly reduce the THP-1 inflammatory factors IL-6 and TNF-α induced by LPS during a simulated weightlessness period. It could inhibit inflammation by decreasing the release of IL-6, TNF-α, IL-1β, and IL-8 during the weightlessness recovery phase

as well. Our study provides a theoretical foundation for further exploring the application of ginsenosides in the prevention of weightless-induced inflammation.

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Competing interests

The authors declare no conflict of interest.

References

[1] SIBONGA JD. Spaceflight-induced bone loss: is there an osteoporosis risk? Current Osteoporosis Reports, 2013, 11(2): 92–98.

[2] STEIN TP. Weight, muscle and bone loss during space flight: another perspective. European Journal of Applied Physiology, 2013, 113(9): 2171–2181.

[3] HUANG B, LI DG, HUANG Y, et al. Effects of spaceflight and simulated microgravity on microbial growth and secondary metabolism. Military Medical Research, 2018, 5(1): 18.

[4] JIRAK P, MIRNA M, REZAR R, et al. How spaceflight challenges human cardiovascular health. European Journal of Preventive Cardiology, 2022, 29(10): 1399–1411.

[5] TAYLOR GR, KONSTANTINOVA I, SONNENFELD G, et al. Changes in the immune system during and after spaceflight. Advances in Space Biology and Medicine, 1997, 6: 1–32.

[6] MERMEL LA. Infection prevention and control during prolonged human space travel. Clinical Infectious Diseases, 2013, 56(1): 123–130.

[7] HIRAYAMA J, HATTORI A, TAKAHASHI A, et al. Physiological consequences of space flight, including abnormal bone metabolism, space radiation injury, and circadian clock dysregulation: implications of melatonin use and regulation as a countermeasure. Journal of Pineal Research, 2023, 74(1): e12834.

[8] CRUCIAN BE, STOWE RP, PIERSON DL, et al. Immune system dysregulation following short- vs long-duration spaceflight. Aviation, Space, and Environmental Medicine, 2008, 79(9): 835–843.

[9] TAYLOR PW, SOMMER AP. Towards rational treatment of bacterial infections during extended space travel. International Journal of Antimicrobial Agents, 2005, 26(3): 183–187.

[10] CRUCIAN B, BABIAK-VAZQUEZ A, JOHNSTON S, et al. Incidence of clinical symptoms during long-duration orbital spaceflight. International Journal of General Medicine, 2016, 9: 383–391.

[11] ZHU LY, NIE L, XIE SS, et al. Attenuation of antiviral immune response caused by perturbation of TRIM25-mediated RIG-I activation under simulated microgravity. Cell Reports, 2021, 34(1): 108600.

[12] DURNOVA GN, KAPLANSKY AS, PORTUGALOV VV. Effect of a 22-day space flight on the lymphoid organs of rats. Aviation, Space, and Environmental Medicine, 1976, 47(6): 588–591.

[13] BAQAI FP, GRIDLEY DS, SLATER JM, et al. Effects of spaceflight on innate immune function and antioxidant gene expression. Journal of Applied Physiology, 2009, 106(6): 1935–1942.

[14] SHI JX, WANG YF, HE JA, et al. Intestinal microbiota contributes to colonic epithelial changes in simulated microgravity mouse model. FASEB Journal, 2017, 31(8): 3695–3709.

[15] JIANG M, WANG HM, LIU ZF, et al. Endoplasmic reticulum stress-dependent activation of iNOS/NO-NF-κB signaling and NLRP3 inflammasome contributes to endothelial inflammation and apoptosis associated with microgravity. FASEB Journal, 2020, 34(8): 10835–10849.

[16] KIM JH. Pharmacological and medical applications of *Panax ginseng* and ginsenosides: a review for use in cardiovascular diseases. Journal of Ginseng Research, 2018, 42(3): 264–269.

[17] LIU JQ, NILE SH, XU GL, et al. Systematic exploration of *Astragalus membranaceus* and *Panax ginseng* as immune regulators: insights from the comparative biological and computational analysis. Phytomedicine, 2021, 86: 153077.

[18] GUI QF, XU ZR, XU KY, et al. The efficacy of ginseng-related therapies in type 2 diabetes mellitus: an updated systematic review and meta-analysis. Medicine, 2016, 95(6): e2584.

[19] ONG WY, FAROOQUI T, KOH HL, et al. Protective effects of ginseng on neurological disorders. Frontiers in Aging Neuroscience, 2015, 7: 129.

[20] MAJEED F, MALIK FZ, AHMED Z, et al. Ginseng phytochemicals as therapeutics in oncology: recent perspectives. Biomedicine & Pharmacotherapy, 2018, 100: 52–63.

[21] ZONG Y, YU WH, HONG HH, et al. Ginsenoside Rg1 improves inflammation and autophagy of the pancreas and spleen in streptozotocin-induced type 1 diabetic mice. International Journal of Endocrinology, 2023, 2023: 3595992.

[22] LUO M, YAN DS, SUN QS, et al. Ginsenoside Rg1 attenuates cardiomyocyte apoptosis and inflammation via the TLR4/NF-kB/NLRP3 pathway. Journal of Cellular Biochemistry, 2020, 121(4): 2994–3004.

[23] LIU XX, MI XJ, WANG Z, et al. Ginsenoside Rg3 promotes regression from hepatic fibrosis through reducing inflammation-mediated autophagy signaling pathway. Cell Death & Disease, 2020, 11(6): 454.

[24] BAI X, FU RZ, DUAN ZG, et al. Ginsenoside Rh4 alleviates antibiotic-induced intestinal inflammation by regulating the TLR4-MyD88-MAPK pathway and gut microbiota composition. Food & Function, 2021, 12(7): 2874–2885.

[25] XU YQ, LV W, WU HJ, et al. Ginsenoside regulates Treg/Th17 cell ratio and inhibits inflammation to treat COPD. Die Pharmazie, 2020, 75(11): 590–594.

[26] CHANPUT W, MES JJ, WICHERS HJ. THP-1 cell line: an *in vitro* cell model for immune modulation approach. International Immunopharmacology, 2014, 23(1): 37–45.

[27] EIERMANN P, KOPP S, HAUSLAGE J, et al. Adaptation of a 2-D clinostat for simulated microgravity experiments with adherent cells. Microgravity Science and Technology, 2013, 25(3):

153–159.

[28] PAVLETIĆ B, RUNZHEIMER K, SIEMS K, et al. Spaceflight virology: what do we know about viral threats in the spaceflight environment? Astrobiology, 2022, 22(2): 210-224.

[29] CHEN Y, XU CY, WANG P, et al. Effect of long-term simulated microgravity on immune system and lung tissues in rhesus macaque. Inflammation, 2017, 40(2): 589–600.

[30] LI X, WEI SZ, NIU SQ, et al. Network pharmacology prediction and molecular docking-based strategy to explore the potential mechanism of Huanglian Jiedu Decoction against sepsis. Computers in Biology and Medicine, 2022, 144: 105389.

[31] ZHAO JQ, LV TW, QUAN JJ, et al. Identification of target genes in cardiomyopathy with fibrosis and cardiac remodeling. Journal of Biomedical Science, 2018, 25(1): 63.

[32] LOPEZ-CASTEJON G, BROUGH D. Understanding the mechanism of IL-1β secretion. Cytokine & Growth Factor Reviews, 2011, 22(4): 189–195.

[33] ROSE-JOHN S, JENKINS BJ, GARBERS C, et al. Targeting IL-6 trans-signalling: past, present and future prospects. Nature Reviews, Immunology, 2023, 23(10): 666–681.

[34] ALOLGA RN, NUER-ALLORNUVOR GF, KUUGBEE ED, et al. Ginsenoside Rg1 and the control of inflammation implications for the therapy of type 2 diabetes: a review of scientific findings and call for further research. Pharmacological Research, 2020, 152: 104630.

[35] MECHESSO AF, QUAH Y, PARK SC. Ginsenoside Rg3 reduces the adhesion, invasion, and intracellular survival of Salmonella enterica serovar Typhimurium. Journal of Ginseng Research, 2021, 45(1): 75–85.

[36] ZHANG DQ, HAMDOUN S, CHEN RH, et al. Identification of natural compounds as SARS-CoV-2 entry inhibitors by molecular docking-based virtual screening with bio-layer interferometry. Pharmacological Research, 2021, 172: 105820.

[37] CHEN Z, WANG G, XIE X, et al. Ginsenoside Rg5 allosterically interacts with P2RY(12) and ameliorates deep venous thrombosis by counteracting neutrophil NETosis and inflammatory response. Frontiers in Immunology, 2022, 13: 918476.

[38] HAN SK, JOO MK, KIM JK, et al. Bifidobacteria-fermented red ginseng and Its constituents ginsenoside rd and protopanaxatriol alleviate anxiety/depression in mice by the amelioration of gut dysbiosis. Nutrients, 2020, 12(4): 901.

[39] SCHWABE RF, SCHNABL B, KWEON YO, et al. CD40 activates NF-kappa B and c-Jun N-terminal kinase and enhances chemokine secretion on activated human hepatic stellate cells. Journal of Immunology, 2001, 166(11): 6812–6819.

[40] KIM JE, KANG TC. PKC, AKT and ERK1/2-mediated modulations of PARP1, NF-κB and PEA15 activities distinctly regulate regional specific astroglial responses following status epilepticus. Frontiers in Molecular Neuroscience, 2019, 12: 180.

[41] ZHAO CL, CAI XP, WANG Y, et al. NAT1 promotes osteolytic metastasis in luminal breast cancer by regulating the bone metastatic niche via NF-κB/IL-1B signaling pathway. American Journal of Cancer Research, 2020, 10(8): 2464–2479.

[42] MENG M, KLINGENSMITH NJ, COOPERSMITH CM. New insights into the gut as the driver of critical illness and organ failure. Current Opinion in Critical Care, 2017, 23(2): 143–148.

[43] NIGHOT M, AL-SADI R, GUO SH, et al. Lipopolysaccharide-induced increase in intestinal epithelial tight permeability is mediated by toll-like receptor 4/myeloid differentiation primary response 88 (MyD88) activation of myosin light chain kinase expression. The American Journal of Pathology, 2017, 187(12): 2698–2710.

人参皂苷对模拟失重巨噬细胞的保护作用研究

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【摘要】目的 探究失重条件下炎症的作用机制及人参皂苷对巨噬细胞的炎症保护作用。**方法** 首先通过GeneCards数据库检索与失重、炎症和免疫相关的基因，再通过基因/蛋白相互作用检索搜查工具（STRING）蛋白网络分析，寻找失重诱导炎症的核心靶点。通过非标记定量蛋白组学（LFQ）寻找人参皂苷处理人单核细胞白血病（THP-1）细胞的差异基因，通过基因本体（GO）、京都基因与基因组百科全书（KEGG）富集分析研究人参皂苷主要参与的生物过程和信号通路及人参皂苷针对失重性炎症的主要作用靶点。最后采用酶联免疫吸附试验（ELISA）法检测人参皂苷对脂多糖（LPS）诱导的THP-1细胞模拟失重期及失重恢复期白细胞介素（IL）-1β、IL-6、IL-8和肿瘤坏死因子α（TNF-α）的分泌影响。**结果** 通过GeneCards数据库检索到炎症相关基因2 933个，失重相关基因425个，免疫相关基因4 564个。通过蛋白相互作用网络（PPI）确定了与失重诱导的炎症相关的靶点，如IL-1β、IL-6、TNF-α和白蛋白（ALB）。通过蛋白组学研究发现人参皂苷主要参与多种炎症相关信号通路及病原微生物感染通路，并显著改变CD40、IL-1β和腺苷二磷酸核糖聚合酶（PARP1）等蛋白表达。细胞模拟失重试验显示人参皂苷可以显著降低模拟失重期LPS诱导单核/巨噬细胞THP-1炎性因子IL-6、TNF-α的分泌（$P < 0.000\,1$），同时还可降低失重恢复期IL-1β、IL-6、IL-8和TNF-α的分泌（$P < 0.000\,1$）。**结论** 失重可影响多种炎症相关信号通路，而人参皂苷可减少模拟失重巨噬细胞多种炎性因子的分泌起到缓解炎症的作用。本研究为进一步探究人参皂苷应用于防护失重条件下LPS诱导的炎症提供理论基础。

【关键词】模拟失重；人参皂苷；炎症因子；蛋白组学；生物标志物