National Report on Space Medicine Progress in 2012–2014

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ABSTRACT

The application of manned spaceflight makes a great impetus on the development of space medicine. With the deep growth of Chinese manned space, our national space medicine has attained great achievements. In this paper, the basic research of Space Medicine and its application in China during 2012–2014 were briefly reviewed.

KEY WORDS

Spaceflight, Space medicine, Progress

1 Introduction

In China, Space Medicine has achieved great development since 2012. After the rendezvous and docking mission, many new realizations has been acquired to basic regularity of the major medical issues during long term space flight, which promoted the development of spaceflight engineering, and provides an important support for the building of China Space Station.

In this paper, the basic research of Space Medicine and its application in China during 2012–2014 are briefly reviewed.

2 Astronaut Health Ensurance Technology

In the rendezvous and docking missions, the technology for astronaut health maintenance in-orbit was achieved. Astronaut health care system was created, including crew medical monitoring and treatment, traditional Chinese medicine prevention, mental maintenance, countermeasure and protection of physiological effects of weightlessness, food and nutrition provision, psychological research *etc*. It provided effective supports for astronauts' living and working conditions in-orbit.

2.1 Medical Monitoring and Treatment

The crew health care techniques in long term spaceflight were preliminarily explored. A number of space medicine research techniques were tested and verified. After a series of Head-down Bed Rest experiments (HDBR) and spaceflights missions, lots of astronaut's data in microgravity and simulated weightlessness environment were acquired, and astronauts databases were founded, including physiological function, endocrine function, biochemistry indexes, which provided important supports for astronauts' medical monitoring and treatment in-orbit.

(1) A series of medical techniques were verified in HDBR and parabolic flight experiment, including dynamic electrocardio-monitoring, non-trauma heart function monitoring, motion lung-function examination, and medical biochemical monitoring, *etc*., which have become the fundamental methods to support and evaluate the astronauts health in the rendezvous and docking mission.

(2) A 15-day HDBR with female project was organized to evaluate, compare and refine female countermeasure in simulated weightlessness. Obtained data were used for the health care of female astronauts in the Shenzhou-9 and Shenzhou-10 missions.

(3) Experiments were performed to study the influence of weightlessness on emotion, cognition of sleep deprivation, confinement, and to explore effective mental countermeasures. Concerning anxiety, depression and reaction time, there are no significant changes in isolations only, but Sleep Deprivation affects them negatively. Sleep Deprivation influences the performance of simple operations, but not the performance of complex operations. Group mental training method can effectually antagonize the negative effect of Sleep Deprivation.

(4) One study analyzed the contour chart of blood flow pressure, extreme pressure and its position to quantify the Distribution of Blood Flow Pressure (DBFP) in thirteen different postures with gravity considered or not ($g \neq 0$ or $g = 0$). The aim was to determine the suitable body positions, in which the postural model of a single vessel could be simplified to two-dimensional (2D) symmetrical one while only considering such factors as posture and gravity. Computational fluid dynamic simulations were performed. Numerical results demonstrated that the DBFP showed 2D axisymmetry at ± 90° and three-dimensional (3D) asymmetry at any other posture with $g \neq 0$, and 2D axisymmetrical one at any posture with $g = 0$. Therefore, establishing a 2D model of a vessel is feasible in space and at ± 90° posture on Earth. In addition, the maximum pressure occurred between the inlet and the middle of the vessel, and its position variation mainly happened in the range of 0°–15°. For a single vessel, this study provides the first theoretical evidence for cardiovascular modeling in microgravity and may help guide the researchers in designing.

2.2 Microgravity Physiological Effects and Countermeasures

Aiming at extended duration female astronaut involved rendezvous and docking mission, several experiments were carried out including 30-day HDBR, the woman 15-day HDBR and 60-day HDBR. Through these experiments, the effects of integrated countermeasures were validated, containing thigh cuff, penguin suit, Lower Body Negative Pressure device (LBNP), bicycle ergometer and expander. The application of the integrated countermeasures in extended duration and multi-physiological systems supported the rendezvous and docking mission.

There was an experiment to quantify the impact of 60-day Head-Down Bed Rest (HDBR) with counter-measures on arterial and venous response to tilt. Results showed that at post-HDBR tilt, the cerebral (MCA) decreased more compared with pre-HDBR tilt in the control (Con), the Resistive Vibration Exercise (RVE), and Herb groups, the MCA/the femoral (FEM) tended to decrease in the Con and Herb groups (not significant) but remained stable in the RVE gr. leg vascular resistance (FRI) dropped in the Con gr., but remained stable in the Herb gr. and increased in the RVE gr. the Portal Vein section (PV) decreased less in the Con and Herb groups but remained unchanged in the RVE gr. MCA/PV decreased in the Con and Herb groups, but increased to a similar extent in the RVE gr. Gast section significantly increased more in the Con gr. only, whereas Tib section increased more in the Con and Herb groups but not in the RVE gr. The percent change in Saph

section was similar at pre- and post-HDBR tilt. So in the Con gr., vasoconstriction was reduced in leg and splanchnic areas. RVE and Herb contributed to prevent the loss of vasoconstriction in both areas, but the effect of RVE was higher. RVE and Herb contributed to limit Gast distension whereas only RVE had a protective effect on the Tib.

RVE platform was studied to test if an intervention RVE protocol would be effective to protect bed rest-induced bone loss. Results showed that RVE significantly prevented bone loss at multiple skeletal sites, including calcaneus, distal tibia, hip, and lumbar spine (L2–L4). The ratio of urinary calcium and creatinine was found higher after starting bed rest in CON group while no significant changes were observed in RVE group. No significant temporal change was found for osteocalcin-N during and after bed rest in CON group. However, a significant increase was shown after bed rest in RVE group. In both groups, the urinary concentration of bone resorption markers, such as C-telopeptide of Type I collagen (CTX-I) and Deoxypyridinoline (DPD), were significantly elevated after bed rest. In the CON group, no significant temporal effect was found for Hydroxyproline (HOP), CTX-I, and DPD during bed rest and the serum concentration of HOP and TGF-β significantly increased about 52.04% and 24.03%, respectively only after bed rest. However, all these markers tended to decrease in the RVE group. So the intervention of RVE retarded bone loss induced by simulated microgravity in humans that was mainly attributed to its anabolic effects.

An advanced zero-gravity locomotion stimulated system was established combined with cardiopulmonary function test system, isokenic strength test system, foot force test system and biomechanics test system. In this system, the leg motion compensation apparatus counterbalance to the running leg's gravity. The subject force loading system can provide loading force similar to 1 g. The system is applied for designing and evaluating the space treadmill, and used for exercise method and biomechanics research.

With the systematictic biology methods and technology, mechanism of physiological effects induced by weightlessness were researched including cardiovascular deconditioning, vestibular dysfunction, bone loss, muscle atrophy, immunology dysfunction and so on. Through these studies, physiology adaptation and readaptation theory in different gravity was built and provides theory basis for the development of the countermeasure against physiological deconditioning.

2.3 Nutrition and Metabolism Research

The intakes of chief dietary nutriments for female volunteers during 15-day HDBR were analyzed. Results showed that intakes of protein were higher than standard, the intakes of fat were sufficient whereas the intakes of carbohydrate were insufficient. The intakes of energy for control group increased along with the time of bed rest, while the intakes of energy for Lower Body Negative Pressure Group (LBNP) and lower body negative pressure association with bicycle as loaded exercise group (ERGO+LBNP) decreased or did not change. The intakes of energy for female volunteers during HDBR were sufficient, but constitutes of nutriments were not proper enough.

The effects of the compounding nutrients on bone metabolism in tail-suspended rats were researched. The designed nutrient compound prescription was optimized with uniform design method. Results showed the biological mechanical, bone density and blood biochemical of bone metabolism changes induced by simulated weightlessness were ameliorated by the optimized nutrient compounds. Most of their amelioration was similar to other positive control drugs, even better in a few indices. It can be concluded that the bone loss of rats induced by simulated weightlessness is ameliorated by the optimized compounding nutrients.

3 Cellular and Molecular Research

Space cellular and molecular biology is to study the mechanism of changes which affect astronaut's health such as bone loss and muscle dystrophy, heart and lung disorder, immunity system inhibition and alteration in motor sensory system. The aim is to disclose intracellular changes under space environment.

The scientific issues for the long term space-flight medicine were preliminarily explored. A large amount of cellular and molecular studies have been performed by onboard and ground experiments. They provide powerful support and important basis for the further development of space medicine.

We proposed a new hypothesis on the physiological effects, response to gravity mechanism and countermeasures technology in spaceflight, which is organ-organ interaction mechanism of bone loss induced by microgravity; recognized three aspects of orthostatic stress induce by long term bed rest; proposed a new mechanism for transduction of mechanical and cytoskeleton and a new

view for nitrated proteins being specific biomarker for oxidative stress and injury; found single-methylated histone in the radiation induced damage and repair research; found a new microRNA which could regulate bone loss induced by microgravity and age. All these theory and experimental results about cardiovascular alterations in spaceflight and the effect of cytokine in response to gravity can support long-term human presence, development and exploration of space. The results have been already published in a series of articles in international academic journals, especially the paper "miR214 targets ATF4 to inhibit bone formation'' published in *Nature medicine* in January 2013. The weightlessness physiological effect monitor device and space automatic cell culture system were also provided, which can support the building of China Space Station.

- We studied cytoskeleton and integrin involved in the regulation of osteoblasts differentiation in space induced bone loss; explored a novel pathway for circulating microRNA in the differentiation of osteoclasts and crosstalk with osteoblasts.
- As for the molecular mechanisms that mediate the microgravity-induced muscle atrophy, we found that increased TGF-β1, IL-1 and IL-18 showed significant correlations with the formation of microgravity-induced muscle atrophy by exploring human bed-rest experimental data, as well as decreased MCP-2. We also demonstrated that Smad3 could be transported into the nucleus and regulate the transcription of type IIa gene after being phosphorylated. Salidroside could selectively inhibit the TGF-β1/Smad3 signaling pathway, which is critical in triggering and mediating the progress of microgravity-induced muscle atrophy.
- We identified and validated protein-coding ceRNAs may be useful for discovering potential ceRNAs for mRNAs of interest in different pathologies of heart disease.
- To construct a stable expression of Runx2 in C2C12, MG63 cells, and primarily investigate the role of Runx2 against bone loss induced by spaceflight, myoblast C2C12 and pre-osteoblast MG63 was transfected with Runx2 and selected overexpressing cell clone with G418. The stable cell lines and untransfected cell lines were cultured in clinostat to simulate microgravity condition. The mRNA expression level of Alkaline Phosphatase (ALP) and CollaI increased in C2C12-Runx2 and MG63-Runx2 cells, and decreased in the chnorotation group compared with the control group. The decreased degree of ALP, CollaI mRNA expression in both transfected cell lines was lower than in untransfected cell lines under simulated microgravity condition. The data from clinorotation and real-time quantitative PCR suggest that Runx2 can partly antagonize the decreased expression of osteogenic specific molecules induced by simulated microgravity.
- We investigated the involvement of microfilament in mediating the effects of microgravity and BMP2 induction on Cbfa1 activity. For this purpose we constructed a fluorescent reporter cell line (OSE-MG63) of Cbfa1 activity by stably transfecting MG63 cells with a reporter consisting of six tandem copies of OSE2 and a minimal mOG2 promoter upstream of Enhanced Green Fluorescent Protein (EGFP). The fluorescence intensity of OSE-MG63 showed responsiveness to bone-related cytokines (IGF-I, vitamin D3 and BMP2) and presented an accordant tendency with Alkaline Phosphatase (ALP) activity. Simulated microgravity inhibited Cbfa1 activity, affected the responsiveness of Cbfa1 to cytokine BMP2, and caused a thinning and dispersed distribution of microfilament. Under normal gravity, cytochalasin B significantly attenuated BMP2 induction to Cbfa1 activity as well as DNA binding activity of Cbfa1 to OSE2. The addition of JAS reversed the inhibitory effects of microgravity on the responsiveness of Cbfa1 to BMP2. These results suggest that actin microfilament participates in BMP2's induction to Cbfa1 activity and that its disruption might be an important contributor to microgravity's inhibition on BMP2's osteogenic induction.
- We investigated the post-transcriptional regulation of SOD2 (Superoxide Dismutase 2). MicroRNAs (miRNAs) are 21–25 nt (nucleotide) small noncoding RNAs that have emerged as indispensable regulators of gene expression. It was showed that miR-146a, a widely expressed miRNA, is up-regulated by H2O2-induced stress. By sequence analysis it was found a binding site for miR-146a in the SOD2 mRNA 39UTR, and a luciferase reporter assay confirmed that miR-146a can interact with this SOD2 regulatory region. Our results further show that miR-146a could down-regulate the SOD2 protein expression, and antisense-miR-146a could reverse the decrease of both the SOD2 level and cell viability in H2O2 treated PC12 cells. In conclusion, here we have identified a novel function of miR-146a in the post-transcriptional regulation of SOD2 expression.

4 Environmental Medicine Research

Further understandings were acquired for the impact of space environment on human's physiological status, which provide powerful support and important basis to the measurements research for space medicine and the subsequent Space Station mission engineering design.

(a) With 60 days combined environmental factors

effects experiment, we validated the medicine requirements of harmful gas, noise, electromagnetic radiation in crew modules for long term spaceflight, evaluated the synthetic effects of these factors and studied the protective measures. Results provide supports for medicine requirement and evaluating technique in long term spaceflight.

(b) Trace chemical contaminant generated by human metabolism is a major source of contamination in spacecraft crew module. Expired air, skin gas, and sweat of subjects were analyzed at different exercise states in a simulated module. The exercise states were designed according to the basic activities in the orbit of astronauts. As physical load increased, the concentrations of chemical compounds from human skin and expired air increased correspondingly. The species and the off-gassing rates of pollutants from human metabolism are different among Chinese, Americans and Russians due to different ethnicity and dietary customs. This research provides data to aid in the design, development and operation of China's long duration space mission.

(c) There were some experiments to study the mechanism of space radiation effects on human body, and to find effective protection measures. In the rendezvous and docking mission, radiation effects on human lymphocyte were researched, and calculation model of space radiation dose was established which was applied to the biological mechanism of space radiation to explore measures of space radiation monitoring and protection on-orbit.

(d) It has been a focus topic to develop low-toxic, effective, and practical radiation preventor from China's abundant medical herb resources. This is a new orientation and trend for the space radiation protection. There were experiments to investigate the effects of Pueraria Lobata Flower Polysaccharide (PLFP) on X-irradiated mice and research the anti-radiation effects of PLFP on irradiation-damaged mice. It has been proved that PLFP can reduce the radiation damage, whose mechanism of action may be relevant to enhancing the oxidative resistance and DNA protection.

(e) The antioxidant and immune protective effect of glycosides (aglycone) in Rhodiola were investigated for its application in radiation protection. In vitro antioxidant models and radiation-induced cell damage model were established to find its anti-radiation active ingredients. In addition, using Caco-2 cell model, the permeability and mechanism of transportation of the anti-radiation active ingredients were investigated. Their potential as a radiation protective agent was evaluated. The results suggest that Salidroside, Tyrosol, Rosavin and Rosarin are anti-radiation active ingredients in Rhodiola.

5 Experiments In-orbit

In the rendezvous and docking mission, for the first time, crew carried out space medicine experiments on a large scale, and acquired many important results.

These studies belong to four research fields.

(i) Techniques of astronauts' medical monitoring and hearth maintenance.

(ii) Effects and mechanism of microgravity on human.

(iii) Requirements and evaluation of medicine of the spacelab design.

(iv) Astronaut space adaptability and space operation ergonomics study.

With the basic research of space medicine and the verification by spaceflight, breakthroughs were made in a number of key technologies for in-orbit experiments. Consequently, series of innovative scientific outcomes of basic research in space medicine were achieved, and a number of space experiment platform techniques were tested and verified.

(a) We systematically acquired a lot of basic data of astronaut's physiological effect, nutrition and metabolism, mental state, which could help us to understand the changing characteristics of human cardiopulmonary function, muscle-skeleton function, nutrition and metabolism level, brain function, biological rhythm, psychological state, *etc*. These data supported to evaluate the crew health state and develop better astronaut health maintenance technology.

(b) Methods of cognition and hand-eyes concordant test were established in-orbit, a lot of basic data about astronaut's work ability were acquired, and the changing characteristics were analyzed. Results would help the crew work design in-orbit and human factor engineering research to increase the crew achievement.

(c) Aiming directly at the bone loss in long-term spaceflight, a new method to resist space bone loss was validated in-orbit. This method could increase the level of bone density and metabolism. So the method would be an effective countermeasure which could be applied in long-term spaceflight.

(d) Environmental medicine is an important research field. A series of environ- mental monitoring technology were applied in-orbit to acquire various data of harmful gas, noise and other environmental factors, which supported the evaluation of Tiangong-1 Spacelab environment, and would support the engineering design of China Space Station.

(e) A series of space medicine equipment were developed to explore physiological effects and gravity responding mechanism, provide test platform to carry out prospective

and groundbreaking space medicine experiments in-orbit.

Integrated with many groundbreaking techniques such as optics, hydrodynamics, microtronics, we have overcome the key problem of picking up weak physiological signals in complicated electromagnetic environment and cell culture controlling in microgravity environment, developed a series of physiological and cellular experiment equipment, it would provide a basic platform for the experiments in China Space Station.

6 Considerations for Future

Though great progresses in space medicine have been achieved during recent years, the challenges on long-term spaceflight medical problems are also prominent with the starting of China Space Station plan.

Focused on the physiological, psychological and behavioral capacity problems, a highly open space experiment platform will be established in China Space Station, which supports the researches on the health monitoring and treatment technology, countermeasures to microgravity effects and space radiation effects, crew behavioral capacities, traditional medicine, and so on.

The ongoing goals of manned space-medico include: (i) to master basic techniques for long term living in low Earth orbit, to solve the major medical issues during long term space flights, and to achieve healthy living and effective work for long term in-orbit missions; (ii) to establish the national space laboratory, to develop the space science and application capability at international level, to carry out large scale space scientific and technological experiments, and to obtain the achievements with great scientific value.

REFERENCE

[1] Wang X G, Li Q, Lü K, *et al*. MiR-214 targets ATF4 to inhibit bone formation. Nature Medicine [J]. *Nat. Med*., 2013, **19**: 93-100

[2] Wang H, Wan Y, Tam K F, Ling S, Bai Y, Deng Y, Liu Y, Zhang H, Cheung W H, Qin J, Cheng L C, Leung K S, Li Y. Resistive vibration exercise retards bone lossin weight-bearing skeletons during 60 days bed rest [J]. *Osteoporos Int*, 2012, **23**: 2169-2178

[3] Yuan M, Coupé M, Bai Y, Gauquelin-Koch G, Jiang S, Aubry P, Wan Y, Custaud M A, Li Y, Arbeille P. Peripheralarterial and venousresponse to tilt test after a 60-day bedrest with and without countermeasures (ES-IBREP). *PLoS One*, 2012, **7**(3): 32854

[4] Gu Yin, Tan Yingjun, Wang Chunyan, Nie Jielin, Yu Jianru, Li Yinghui. A surface plasmon resonance sensor platform coupled with gold nanoparticle probes for unpurified nucleic acids detection [J]. *Anal. Lett.*, 2012, **45**: 2210-2220

[5] Feima Guo, Zhongquan Dai, Feng Wu, *et al*. Gravity affects the responsiveness of Runx2 to 1, 25-dihydroxyvitamin D3 (VD3) [J]. *Acta Astron*., 2013, **84**: 189-196

[6] Jing Liu, Yuanhua Peng, Zhiwei Cui, *et al*. Depressed mitochondrial biogenesisand dynamic remodeling in mouse tibialis anterior and gastrocnemius induced by 4-week hindlimb unloading [J]. *IUBMB Life*, 2012, **64**(11): 901-910

[7] Mu Wenying, Chen Shanguang, Zhuang Fengyuan, Li Yinghui, Li Yu. Quantification of the distribution of blood flow pressure with postures [J]. *J. Biomed. Sci. Eng.*, 2012, **5**: 113-119

[8] Dai Z Q, Tan Y J, Yang F, Qu L N, Zhang H Y, Wan Y M, Li Y H . Altered actin dynamics and functions of osteoblast-like cells in parabolic flight may involve ERK1/2 [J]. *Microgravity Sci. Tech.*, 2011, **23**(1): 19-27

[9] Dai Zhongquan, Wu Feng, Chen Jian, *et al*. Actin microfilament mediates osteoblast cbfa1 responsiveness to BMP2 under simulated microgravity [J]. *PloS. One*, 2013, **8**(5): 63661

[10] Ji Guohua., Lü Ke, Chen Hailong, *et al*. MiR-146a Regulates SOD2 Expression in H2O2 Stimulated PC12 Cells [J]. *PloS One*, 2013, **8**(7): 69351

[11] Guo Feima, Dai Zhongquan, Wu Feng, *et al*. Gravity affects the responsiveness of Runx2 to 1, 25-dihydroxyvitamin D3 (VD3) [J]. *Acta Astron*., 2013, **84**: 189-196

[12] Ling Shukuan, Sun Qiao, Li Yuheng, Zhang Luo, *et al*. CKIP-1 Inhibits Cardiac Hypertrophy by Regulating Class II Histone Deacetylase Phosphorylation Through Recruiting PP2A [J]. *Circulation*, 2012,**126**: 3028-3040

[13] Guo Lihua, He Xinxing, Ding Junping, *et al*. Investigation of volatile organic compounds outgassed from bakelite at different temperatures using gas chromatography-mass spectrometry[J]. *J. Beijing Univ. Chem. Tech.:Nat. Sci*., 2012, **39** (1): 21-23

[14] Ming Yuan, Mickael Coupe, Yanqiang Bai, *et al*. Peripheral arterial and venous response to tilt test after a 60-day bedrest with and without countermeasures (ES-IBREP). *PLoS One*, 2012, **7**(3): 32854

[15] Mu Wenying, Chen Shanguang, Zhuang Fengyuan, *et al*. Quantification of the distribution of blood flow pressure with postures [J]. *J. Biomed. Sci. Eng.*, 2012, **5**: 113-119

[16] Wang H, Wan Y, Tam S K F, *et al*. Resistive vibration exercise retards bone loss in weight-bearing skeletons during 60 days bed rest [J]. *Osteoporos Int*, 2012, **23:** 2169-2178