Bone Protection Effects of A Novel Chinese Herbal Formula, Taikong Yangxin Prescription (太空养心方), in Hindlimb Unloaded Rats against Bone Deterioration*

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ABSTRACT Objective: To investigate the protective effects of a Chinese herbal formula, Taikong Yangxin Prescription (太空养心方, TKYXP) against bone deterioration in a hindlimb unloaded (tail-suspension) rat model. Methods: Thirty-two male Sprague-Dawley rats were divided into 4 groups: tail-suspension group fed with 2.5 g·kg⁻¹·day⁻¹ of TKYXP extract (high dose), tail-suspension group fed with 1.25 g·kg⁻¹·day⁻¹ (low dose), tail-suspended group treated with water placebo (placebo control group) and non tail-suspended group. The effects of TKYXP on bone were assessed using peripheral quantitative computed tomography (pQCT), micro-computerized tomography (micro-CT) and three-point bending biomechanical test on the femur in vivo. Results: TKYXP had a significant protective effect against bone loss induced by tail-suspension on day 28, as shown in the reduction in bone mineral density (BMD) loss, preservation of bone micro-architecture and biomechanical strength. The administration of high dose TKYXP could significantly reduce the total BMD loss by 4.8% and 8.0% at the femur and tibia regions, respectively, compared with the placebo control group (P<0.01) on day 28. Its bone protective effect on the femur was further substantiated by the increases of the trabecular BMD (by 6.6%), bone volume fraction (by 20.9%), trabecular number (by 9.5%) and thickness (by 11.9%) as compared with the placebo control group. Conclusion: TKYXP may protect the bone under weightless influence from gradual structural deterioration in the tail-suspension model.

KEYWORDS tail-suspension rat model, osteoporosis peripheral quantitative computed tomography, micro-computerized tomography, Chinese herbal medicine

Spaceflight-related osteoporosis is a well-known result of skeletal unloading in humans during long-duration spaceflights.¹ ² In vivo studies also demonstrated marked bone loss, disruption of bone architecture, and impairment of bone mechanical properties following space flight³ or immobilization by tail-suspension.⁴ A hindlimb unloading tail-suspension model is frequently used in the studies relating to microgravity because it mimics some aspects of exposure to microgravity by eliminating weight-bearing loads from the hindquarters and producing a cephalic fluid shift.⁵ Apart from bone mineral loss, there were a number of reports stated that exposure to weightlessness during space-flight resulted in the reduction of cardiac and immunological functions.⁶ However, up to date, there is a lack of pharmaceutical agents which can effectively prevent the development of such disorders.

Mi, et al⁷ previously demonstrated that a Chinese herbal formula, Taikong Yangxin Prescription (太空养心方, TKYXP) could effectively improve the stroke volume reduction after 5 days in tail-suspension rats. In addition, in a 60-day head-down bed rest clinical trial, Liu, et al⁸ found that TKYXP had an effect on the optimal time delays, and induced faster recovery to the pre-bed rest values. Recently, there was another report stating that TKYXP prevented the loss of vasoconstriction in leg and splanchnic areas after 60-day head-down bed rest treatment.⁹ Apart from its promising cardio-protective...
effects, the information about TKYXP related to the musculoskeletal system is limited. Therefore, we hypothesized that TKYXP can also protect the bone from bone mineral density deterioration and improve the bone quality. To test this hypothesis, we used tail-suspension rats as a model to investigate the effect of TKYXP on the weightless bones using peripheral quantitative computed tomography (pQCT), micro-computerized tomography (micro-CT) and three-point bending biomechanical test after scarifying the rats.

METHODS

Chinese Herbal Medicine

TKYXP was supplied by China Astronaut Research Training Center (CARTC), Beijing, China. The formula prescription was developed by CARTC for astronaut use and microgravity simulation study. This prescription includes less than 10 Chinese herbs. The main medicines are Panax ginseng, Astragali Radix, Ligusticum Wallichi Radix, Schisandra Chinensis Fructus, Ophiopogonis Radix, Rehmanniae Preparata Radix, Drynariae Rhizoma, and Poria Cocos. \(^7\)\(^9\)

Animals and Tail Suspension Treatment Protocol

Thirty-two 3-month old male Sprague-Dawley rats were used in the current study. They were housed individually in a temperature-controlled room (22 ± 2 °C) with 12:12 h dark-light cycle. The rats were provided with standard food chow and water ad libitum throughout the experiment. One-week accommodation period was given to them prior to the start of tail suspension. The protocol of the tail-suspension was modified from that of Morey-Holton. \(^5\) In brief, the tail of each rat was suspended by applying an adhesive tape on its lateral surfaces. The loop of the tape at the tip of the tail was connected to a 360° free rotated hook which was then hanged on an overhead bar at the middle of the top of a cage. The rat was maintained in approximately 35° head-down tilt with its hindlimbs unloaded while its forelimbs were free for crawling. Rats were divided into 4 groups with 8 animals per group: tail-suspended group treated with 2.5 g kg\(^{-1}\)day\(^{-1}\) of TKYXP extract (TKYXP-H); tail-suspended group treated with 1.25 g kg\(^{-1}\)day\(^{-1}\) (TKYXP-L); tail-suspended group treated with water placebo (placebo control group) and non tail-suspended (NTS) groups. Two TKYXP-treated and placebo control groups were suspended for 28 days. For the NTS group rats were not suspended. TKYXP extracts were orally administered to each rat intragastrically for 28 days. No significant weight difference was found among all tail-suspension groups before and after the 28 days of experiment. The experiment was conducted in accordance with the internationally accepted principals for laboratory animal and care.

pQCT

Bone mineral density (BMD) at distal femoral metaphysis and proximal tibial metaphysis of the rat were measured using pQCT (XCT2000, Stratec Medizintechnik GmbH, Germany) at day 0 (before tail-suspension) and day 28. Quality assurance of measurements had been checked by using the hydroxyapatite cone and standard phantoms prior to the scanning of the rats each time. For the BMD measurement, the rat was firstly anesthetized using a cocktail of ketamine and xylazine intramuscularly. It was then fixed on a custom-made translucent plastic holder to ensure a repeatable positioning. Right distal femur and proximal tibia were scanned under the built-in research mode of the pQCT. The scan speed was 25 mm/s with voxel resolution of 0.2 mm. The analytical parameters for trabecular BMD were set as threshold 280 mg/cm\(^3\), contour mode 1 and peel mode 20. The parameters for cortical BMD were set as threshold 551 mg/cm\(^3\) and peel mode 2. BMD at three regions of interest (total and trabecular region) at distal femur and proximal tibia were analyzed separately. Total BMD was the overall BMD of the total cross-sectional area of the bone (including trabecular area). The trabecular bone region was defended by setting an inner area to 35% of the total cross-sectional area. Cortical BMD was the average BMD at the outermost area of the bone where the BMD was over 551 mg/cm\(^3\). The BMD differences on Day 28 from baseline (day 0) within the same group were calculated for cross-sectional comparisons among treatment groups with placebo control groups.

Micro-Computed Tomography

The micro-architecture of the left distal femur was analyzed using a micro-computed tomography (MicroCT 40, Scanco Medical, Switzerland). Briefly, the femur was aligned perpendicularly to the scanning axis. The scanning was conducted at 55 kVp and 144 μA with a resolution of 16 μm per voxel. The trabecular bone within the distal femur was identified with semi-automatically drawn contour at each two-dimensional (2-D) section. Segmentation parameters were fixed at: sigma = 0.5, support = 1.0, and