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Influences of traditional Chinese medicine intervention on the bone growth and metabolism of rats with simulated weightlessness

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ABSTRACT

Objective: To probe into the influences of Chinese herbal compound on the growth and metabolism of weight-bearing bones of tail-suspended rats. **Methods:** Twenty-four male SD rats were randomly divided into blank control group (eight), tail-suspended control group (eight) and Chinese medicine treatment group (eight) according to their weights. No treatment was done for the blank control group. Double distilled water lavage was performed daily for the tail-suspended control group. On the basis of the tail-suspended rat model, the rats were given Chinese herbal compound lavage every day in Chinese medicine treatment group. This compound includes mulberry, *Poria cocos* and barbary wolfberry, *etc.* The test cycle was four weeks. The rats were killed after the experiment. The right femoral bone was taken out for the physical measurements, and the left femoral bone was for the three-point bending test. The influences of Chinese herbal compound on femoral bone growth and biomechanical properties of simulated weightlessness rats were observed. **Results:** (1) After simulated weightlessness (tail-suspension), compared with the blank control group, all the physiological indexes of rat femoral bone decreased in tail-suspended group and Chinese medicine treatment group ($P<0.05$). The strength and rigidity of rat femoral bone decreased in tail-suspended group ($P<0.01$). The maximum load and rigidity coefficient also decreased with the increasing toughness coefficient in the control group ($P<0.01$). (2) After the countermeasure of Chinese herbal compound, each biomechanical indexes showed the tendency of increasing in Chinese medicine treatment group, and theses indexes were close to those of the blank control group ($P<0.05$), which indicated that the bone loss caused by simulated weightlessness was improved. **Conclusions:** Chinese herbal compound for tonifying kidney could effectively prevent the bone loss and have some enhancements on the bone biomechanical properties.

1. Introduction

Various abnormal physiological reactions are prone to appear when the astronauts are in the weightless environment. Data collected by Russian-US scientists indicated that weightlessness have a certain impact on the endocrine secretion, red and white blood corpuscle, inner ear balance organ and bone rarefaction. Disorders of endocrine secretion and inner ear balance organs can be gradually recovered by regulation after gravity recovery. However, no reasonable and effective method has been found for the bone loss and sclerotin fragility caused by weightlessness, the symptom of which is similar to osteoporosis in aged people. Studies have shown that astronauts lost 1%-2% bone weight per month during the flight course[1-3].

At present, most of the prevention methods of osteoporosis are limited to sports, diet and drug therapy. According to the "kidney dominating bones" theory, renal deficiency is the key of osteoporosis. Therefore, it's better to invigorate the kidney in order to strengthen the bone and muscle. A related literature[4] proved that long time microgravity environment may lead to renal deficiency and blood stasis.

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Fu *et al*[5] found that TCM tonifying the kidney can prevent the bone loss after the intervention comparison of Wujia Bugu Formula and alendronate on the bone loss of the simulated weightlessness rats. In this experiment, starting from the overall regulation, we made a compound utilizing self-made tonifying kidney compound and Chinese crude drugs like mulberry, *Poria cocos* and *Barbary wolfberry*.

The protective effects of Chinese herbal compound on bone loss were concluded through the influence of compound preparation on the femoral bone of simulated weightless rats, which provided experimental basis for preventing bone loss by traditional Chinese medicine.

2. Material and methods

2.1. Materials

Twenty-four clean male SD rats were provided by laboratory animal center in our hospital. The rats were randomly divided into blank control group (eight), tail-suspended control group (eight) and Chinese medicine treatment group (eight). The weights were respectively (198.8 ±6.4) g, (202.1±6.9) g and (205.3±5.1) g. During the 4 weeks' experiment, the rats were given adequate clean water and food. The room temperature was kept at (22±2) ℃. The 12 hours of lighting (08:00–20:00) and darkness (20:00–8:00 in the next morning) were alternatively kept.

2.2. Compound

The compound was made of mulberry, *Poria cocos* and *Barbary wolfberry*, *etc*. and worked out by our department. Single Chinese herb was decocted and made into dry powder.

2.3. Modeling

Tail-suspended control group: The rats' tails were fixed by tape, and the distal end of the tape was hung in the high place. The rats were kept in 30 ℃ head-down tilt with forelimb bearing the weight and the hind limb away from the ground, and the rats can freely twist, find food and drink water[6]. The rats were intragastrically administered equal amount of double distilled water every day.

Chinese medicine treatment group: On the basis of tail suspension, the rats were given Chinese medicine lavage every day (100–110 g/ (kg·d), once a day).

Blank control group: The rats were in the state of free activities.

2.4. Methods

Each rat was raised in a single cage. The experiment began after one week adaption. The rats were killed by broking their heads after four weeks. Then, their bilateral femoral bones were removed as soon as possible. The general physical indexes of right femoral bone were measured, and the bone density was calculated. The left femoral bone was sealed in the 4 ℃ environment, and the three-point bending test was performed within 2 days, the biomechanical indicators and bone mineral content were calculated.

Detection of basic physical indexes: including mass (wet, dry, ash content), length, diameter, volume, bone density (calculated according to the mass and volume). The wet mass was measured immediately after taking out the femoral bone. The dry mass can be acquired through heating and baking the sample at 120 ℃ until constant mass. And then, the ash mass was detected after the sample was completely ashed in the 800 ℃ ash stove for 24 hours. The mass was measured by ACA2100 electronic balance (the accuracy is 1.0×10^{-4} g, Denver Instrument Company, USA). The volume was measured by drainage method (the accuracy was 1.0×10^{-4} cm^3). The length and diameter (the thinnest place in middle backbone) were measured by vernier caliper with the accuracy of 0.02 mm.

Biomechanical indexes: The three-point bending test was carried out by Instron 1195 electronic tensile machine (Instron, UK). The sample was put on the machine with the span of 16mm, the loaded weights of 2 kg and 0.05 mm/min loaded speed. The mechanical indexes such as elastic load, maximum load, failure load, limiting deflection, failure deflection, rigidity coefficient and ductility coefficient were calculated according the obtained load-strain curve.

Determination of the bone mineral content: After the three-point bending test, the ash mass and dry mass were detected according to the method in the detection of basic physical indexes. The bone mineral content was the ratio of the two.

2.5. Statistical analysis

All the obtained data were expressed by mean±SD and analyzed by SPSS10.0. After the overall analysis of variance, LSD-*t* test was used for comparison among groups. A $P<0.05$ was taken to indicate a difference of statistical significance.

3. Results

3.1. Changes of body mass

The rat's body mass was measured every week, and the mean value was calculated. After the experiment, the weights of rats in the three groups were (291.7±10.4) g in the blank control group, (254.6±7.5) g in the tail-suspended group and (264.8±6.8) g in the Chinese medicine treatment group, respectively. During the experiment, the body masses of rats in the blank control group increased continuously and were higher than those in both tail-suspended control group and Chinese medicine treatment group. There was no significant difference on the body mass between the latter two groups (Figure 1).

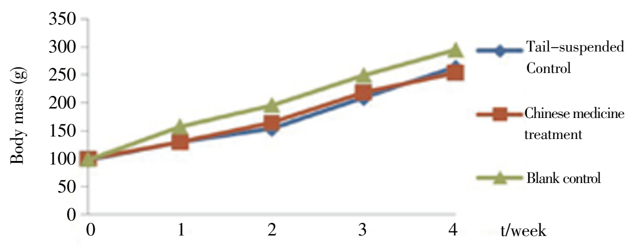

Figure 1. Body mass changes of rats in each group during experimental session.

3.2. Physical index changes of rat femoral bone

After suspension, the body mass (dry, wet), ash content and density of rats in tail–suspended group and Chinese medicine treatment group decreased (P<0.05), but there was no obvious difference in length, which implied that the simulated weightless environment can influence the growth and metabolism of rat femoral bone leading to bone loss. After Chinese medicine treatment, the body mass, diameter and density of rats tended to increased. The above information indicated that TCM compound lavage improved the metabolism of rat femoral bone and can effectively prevent the bone loss in the state of weightlessness (Table 1).

3.3. Mechanical index changes of rat femoral bone

After the processing of suspension, the maximum load and rigidity coefficient of rats decreased, but the ductility coefficient increased in the latter two groups (P<0.01, P<0.01), which indicated that the overall mechanical properties of rat femoral bone obviously decreased because of simulated weightlessness. After the treatment of TCM lavage, the maximum load, rigidity coefficient and elastic load of rats in Chinese medicine treatment group increased in different degrees compared with those in the tail–suspended group, therefore, it can be concluded that the mechanical properties of femoral bone of rats under simulated weightlessness improved after the TCM lavage, which indicated that this Chinese herbal compound preparation can effectively prevent the bone loss in the weightless state.

3.4. Determination of bone mineral content

It is shown in Table 3 that after suspended processing, the dry weight and ashed weight of rats in the latter two groups were lower than those of the blank control group (P<0.01), and the bone mineral content was obviously decreased. After the TCM lavage countermeasure, the bone mineral content was obviously higher than the rats in the tail–suspended group (P<0.05), and there was no significant difference between the blank control group and Chinese medicine treatment group (P>0.05). This indicated that the TCM lavage eased the bone mineral loss.

Table 3

Bone mineral content determination of rats in each group.

Group	Dry weight (mg)	Ashed weight (mg)	Bone mineral content (mg)
Blank control	469.7±42.6	277.1±81.1	0.589±0.005
Tail–suspended control	359.8±30.1^a	190.6±16.2^a	0.529±0.003
Chinese medicine treatment	369.5±42.6	209.6±28.3^a	0.567±0.001

Compared with the blank control group, ^{a}P<0.01.

4. Discussion

The interaction force of each part of human especially the interaction of intracorporal organs should be disappeared

Table 1

Basic physical indexes of rat femur (right) in each group (n=8, mean±SD).

Group	Femoral mass (wet)	Femoral mass (dry) (mg)	Ash content(mg)	Length (mm)	Diameter (mm)	Density (g/cm^3)
Blank control	690.3±49.6	449.7±42.0	287.1± 31.5	31.80±0.91	1.62± 0.09	1.73±0.04
Tail–suspension	586.5±48.2^b	329.8±33.3^b	219.6±18.2^b	30.03±0.97	1.31±0.22^b	1.46±0. 03^b
Chinese medicine treatment	619.4±37.1^b	379.5±32.7	235.6±19.4^b	29.94±0.52	1.51±0.06^a	1.61±0.04^{bc}

Compared with the blank control group, ^{a}P<0.05, ^{b}P<0.01; compared with tail–suspended control group, ^{c}P<0.01.

Table 2

Mechanical indexes of rat femoral bone (left) in each group (n=8, mean±SD).

Group	Elastic load (n)	Maximum load (n)	Elastic deflection (mm)	Maximum deflection (mm)	Rigidity coefficient (N·mm^2·10^4)	Ductility coefficient (mm /N·10^{-2})	Deflection load ratio
Blank control	71.3±14.6	109.9±16.6	0.31± 0.06	0.63± 0.06	2.6± 0.5	1.01±0.17	0.22±0.05
Tail–suspended control	42.5±5.7^b	72.3±9.8^b	0.28±0.08	0.77±0.08^a	1.7±0.4^b	1.65±0.27^b	0.39±0.08^b
Chinese medicine treatment	59.6±7.5^c	89.7±10.3^a	0.30±0.04	0.70±0.07	1.7±0.3^a	1.51±0.19^b	0.30±0.04

Compared with the blank control group, ^{a}P<0.05, ^{b}P<0.01; compared with tail–suspended control group, ^{c}P<0.05.

when simulating the weightless state in the space. Under this condition, the otolith in the human vestibular organ would no longer contact the surrounding nerve cell but directly transmit the signal to central nerve, thus losing the orientating function. Human vestibular organ is closely related to the autonomic nerve system dominating respiration, digestion, circulation, egestion and sudation, *etc*. Therefore, the malfunction of vestibular organ and disappearance of the interaction of human organs would cause dizziness, nausea and vomiting of astronauts. The modeling method used in this experiment is a method successfully simulating the head–distribution of body fluid and the off–load phenomenon of weight–bearing bone in weightlessness by suspending the rats' tail with head down, hind limb hanging in the air but the body can freely rotate, causing the sensory disorder of vestibular organ.

In this experiment, the growth and metabolism condition of bone were judged by detecting the changes of skeletal morphology and biomechanical indexes. The basic physiological indexes indicated that under the simulated weightless environment, the physical form of rat femoral bone changed, the femoral bone density and mineral content decreased, and the growth and metabolism were inhibited. The biomechanical index indicated that the mechanical property of simulated rats significantly decreased, which is conformed to the literatures[7–10].

According to the theory "the kidney being in charge of bone", we main selected the TCM invigorating the kidney and tonifying qi when selecting the compound preparation. This compound contains mulberry, *Poria cocos* and *Barbary wolfberry*, *etc*., having the effect of tonifying the liver and kidney and nourishing yin and blood. This compound can effectively prevent the weakness of waist and knees and bone rarefaction caused by kidney deficiency and splenic asthenia.

Determination results implied that the lavage by Chinese herbal compound in specified dose can improve the femoral bone metabolic abnormality of simulated weightless rats, increase the bone mineral content, strengthen the bone density and effectively prevent the bone loss in weightless state. Therefore, we preliminarily presumed that this compound can improve the bone loss caused by weightlessness, having directive significance in preventing the bone fracture under the weightless state in space. The dosage was not further explored in our experiment. Some experiments indicated that the effect of Chinese compound on preventing bone loss is dose–related, which is, large dose can effectively prevent bone loss, however, the influence of small dose on bone growth and metabolism is not found[11]. These results can be further verified with the help of biochemical index changes of weightless rats and bone tissue sections, and the possible influence of different TCM compounds and administration dosages on the results should also be studied in further studies in order to provide basis for using Chinese compound preparation in preventing bone loss.

Conflict of interest statement

We declare that we have no conflict of interest.

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