Original Article
Effect of epimedium decoction on hemorheology and bone tissue of rabbit steroid induced necrosis of femoral head

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Abstract: Objectives: This study is to research the effect of epimedium decoction on hemorheology, bone mineral density and osteocyte of steroid induced necrosis of femoral head (SONFH). Methods: After establishing the model of SONFH the treatment group was given 10 ml epimedium decoction by gavage. After eight weeks of that, we detected the hemorheology, bone mineral density and osteocyte of rabbits in treatment group, model group and blank group, and made comparison among groups. Results: The results of hemorheology showed that blood viscosity in treatment group was reduced compared with model group. There was a significant difference between the plasma viscosity in treatment group and in model group (P=0.017<0.05). The hematocrit in treatment group was significantly lower than model group (P=1.21×10^{-4}<0.05). Bone mineral density in treatment group was greatly increased compared with model group (P=1.26×10^{-5}<0.05) and without significant difference with blank group. The pathological section indicated that in treatment group most osteocytes in bone trabecula within the femoral head were normal with regular arrangement and visible osteoblast and osteoclast, which was different with model group. And the amount of various hematopoietic cells in cavum medullarein treatment group was increased compared with model group. Conclusion: Epimedium decoction could improve the hemorheology of SONFH, promote the generation of osteoblast and the inhibition of osteoclast, increase bone mineral density, which were beneficial to the rapid repairing of bone tissue.

Keywords: Osteonecrosis of the femoral head (ONFH), bone mineral density, epimedium, hemorheology

Introduction

The incidence of steroid-induced osteonecrosis of the femoral head (SONFH) shows younger trend. Because of the frequent collapse of the femoral head, the hip joint function is impaired, which reduce the quality of life of patients [1]. The specific causes of SONFH are unknown, but some researches reveal that SONFH is related to connective tissue diseases, the usage of steroid hormone during tissue and organ transplantation, and excessive alcohol intake [2]. The main reason is the decrease of blood-supply to bone tissue, which is the commonly development stage of a series of disease [3]. Consequently, patients with long-term treatment of hormone have a remarkably increasing morbidity of osteonecrosis of the femoral head [4]. Over the years, long-term and abundant use of hormone is lack of standardization in clinic. So, patients suffering from osteonecrosis of the femoral head are significantly increased. And at present many therapeutic methods cannot achieve satisfactory results.

Some research has proved that traditional Chinese medicine has special advantages in the treatment and knowledge of SONFH [5, 6]. For example, rhizomadrynariae (RD) is the dry rhizome of drynariafortunei (Kunze) J. Sm from polypodiaceae. The experiment in mice done by Wang WZ et al. showed that RD could regulate immunoreactions, reduce positive T lymphocyte of IL-2 and CD4 in blood, and have anti-inflammatory and anti-proliferative properties [7]. Rhizomadrynariae decoction could block adipogenic differentiation in the process of osteonecrosis of the femoral head [8]. Epimedium sagittatum of berberidaceae is the plant of the epimedium genus, distributed from the Middle East to southeast of China [9]. And epimedium also has anti-inflammatory, anti-
proliferative and anti-tumor properties [10]. In our research, we took interventional treatment on rabbit model of SONFH with epimedium, and investigated the effect and mechanism of epimedium on SONFH.

Materials and methods

Experimental animal and drugs

25 healthy Japanese big-ear rabbits with 1-1.5 kg were selected in this research, fed with ordinary standard food. The rabbits and food were provided by Laboratory Animal Center of Henan Province. Epimedium decoction was prepared by Preparation Room of First Affiliated Hospital of Henan University of Traditional Chinese Medicine. 1080 g epimedium pieces were decocted to 720 ml (with crude drug 1.5 g/ml).

SONFH model

Laboratory Animal Center of Henan Province provided 25 Japanese big-ear rabbits with 1-1.5 kg. After fed for one week, the rabbits were randomly divided into two groups: 8 rabbits in blank group and 17 rabbits in modeling group. All rabbits in modeling group were first injected 10 ml/kg horse serum via the vein of ear margin. After three weeks of that, 6 ml/kg horse serum was injected again via the vein of rabbits' ear margin. After two weeks, 45 mg/kg methylprednisolone were taken intraperitoneal injection once a day for three days consecutively. During the injection of hormone, every day every animal was intraperitoneally injected 10 million unit of penicillin consecutively for seven days for preventing infection. Animals in blank group were fed routinely. After the last injection of hormone, the femoral head section of one rabbit in modeling group was taken pathological examination which confirmed that the model of osteonecrosis of the femoral head succeeded. The remaining 16 animals in modeling group were randomly divided into model group and treatment group. Now that all animals were classified into blank group, model group and treatment group with eight rabbits in each group. After the success of modeling, animals in blank group and model group were given 10 ml normal saline by gavage once a day, and animals in treatment group were given 10 ml epimedium decoction (crude drug 1.5 g/ml) by gavage once a day. All animals were given by gavage for eight weeks.

Determination of hemorheology

After taken medicine for eight weeks, 1 ml/100 mg 10% chloral hydrate were injected to anesthetize animals. 5 ml blood were taken from the vein of ear margin, and then put in the anticoagulant heparin tube with blending upside down. Full-automatic modular hemorrheology meter (Beijing Precil Instrument Co., Ltd., model number: LBY-N6B) was used to detect the hemorrheology indexes (the whole blood high shear viscosity (200 s⁻¹), low-shear viscosity (5 s⁻¹), plasma viscosity, and hematokrit).

Determination of bone mineral density

After anesthetizing with 10% chloral hydrate, the rabbits were dissected in sterile conditions. We observed the appearance, texture, and color and luster of articular cartilage when removing the femoral heads. The side of femoral head was removed muscles. Then the sample was put on bone densitometer loaded automatic bone mineral density measurement software to measure density of femoral head.

Histopathological observation

The other side of femoral head was cut apart along the middle coronal plane. Then the femoral head was fixed in 10% formaldehyde solution for one week, decalcified with 5% nitric acid, and then washed with running water for 24 h. After those, the sample was dehydrated with alcohol. Then the sample was routinely embedded in the paraffin, sectioned, and stained with HE. In each group, the subchondral area was viewed under light microscope to observe the pathological changes of femoral bone tissue and myeloid tissue including bone trabecula, bone marrow hematopoietic tissue, osteoblast, osteocyte and adipocyte.

Statistical analysis

The SPSS17.0 statistical software was adopted to analyze the data. All measurement data were showed as mean ± standard deviation (x ± s). One-way analysis of variance and LSD test were applied to analyze high shear rate, low shear rate, plasma viscosity, hematokrit and bone mineral density among groups. When P was less than 0.05, the differences were statistically significant.
Results

Hemorheology changes

Full-automatic modular hemorrheology meter was applied to test the hemorrheologic changes of animals in the three groups. The results of analysis of variance among blank group, model group and treatment group displayed that there were significant differences in high shear rate ($F=16.976$, $P=0.000$), low shear rate ($F=20.828$, $P=0.000$), plasma viscosity ($F=20.828$, $P=0.000$) and hematokrit ($F=12.715$, $P=0.000$). No matter in high shear rate or low shear rate, the whole blood viscosity in treatment group was obviously lower than the model group ($P=0.031<0.05$). The results of low shear rate showed that there was no significant difference between treatment group and blank group ($P=0.094>0.05$) ($\text{Figure 1A}$ and $\text{1B}$). There were remarkable differences in plasma viscosity of animals in the three groups ($P<0.05$). The results of pairwise comparison revealed that there were obvious differences between treatment group and model group ($P=0.017<0.05$), while there was no obvious difference between treatment group and blank group ($P=0.094>0.05$) ($\text{Figure 1C}$). The results of hematokrit test showed that the hematokrit in treatment group was significantly lower than

Table 1. Bone mineral density detection after taken medicine for eight weeks ($\bar{x} \pm s$)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>BMD (g/cm²)</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank group</td>
<td>8</td>
<td>0.2036$\pm$0.0148</td>
<td>14.007</td>
<td>2.04$\times10^{-5}$</td>
</tr>
<tr>
<td>Model group</td>
<td>8</td>
<td>0.1690$\pm$0.0119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group</td>
<td>8</td>
<td>0.1924$\pm$0.0082</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$F=13.005$, $P=0.000$) and hematokrit ($F=12.715$, $P=0.000$). No matter in high shear rate or low shear rate, the whole blood viscosity in treatment group was obviously lower than the model group ($P=0.031<0.05$). The results of low shear rate showed that there was no significant difference between treatment group and blank group ($P=0.094>0.05$) ($\text{Figure 1A}$ and $\text{1B}$). There were remarkable differences in plasma viscosity of animals in the three groups ($P<0.05$). The results of pairwise comparison revealed that there were obvious differences between treatment group and model group ($P=0.017<0.05$), while there was no obvious difference between treatment group and blank group ($P=0.094>0.05$) ($\text{Figure 1C}$). The results of hematokrit test showed that the hematokrit in treatment group was significantly lower than
Table 2. The results of femoral histopathological slices of white rabbits in three groups

<table>
<thead>
<tr>
<th>Item</th>
<th>Blank group</th>
<th>Model group</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface of femoral head cartilage</td>
<td>Smooth</td>
<td>Slightly rough</td>
<td>Smooth</td>
</tr>
<tr>
<td>Subchondral vessel</td>
<td>Ditissimus</td>
<td>Sparse</td>
<td>Ditissimus</td>
</tr>
<tr>
<td>Chondrocyte morphology</td>
<td>Orderly</td>
<td>Hypertrophic, irregularly arranged</td>
<td>Relatively orderly</td>
</tr>
<tr>
<td>Bone trabecula inside femoral head</td>
<td>Regular, compact and orderly</td>
<td>Sparse, atrophic and incompletely separated</td>
<td>Relatively orderly</td>
</tr>
<tr>
<td>Osteoblast and osteoclast</td>
<td>Osteoblast and little osteoclast</td>
<td>Few osteoblast and many osteoclast</td>
<td>Osteoblast and osteoclast, significant differences with model group</td>
</tr>
<tr>
<td>Hematopoietic cell in cavum medullare</td>
<td>Ditissimus, eumorphism</td>
<td>Reduced</td>
<td>More than model group</td>
</tr>
<tr>
<td>Intramedullary adipocytes</td>
<td>Little adipocytes without cell fusion</td>
<td>Hypertrophic and hyperplastic, large fat droplet</td>
<td>Slightly hyperplastic and hypertrophic, no large fat droplet and cell fusion</td>
</tr>
</tbody>
</table>

Figure 2. The results of bone tissue pathological slices of white rabbits in three groups. Note: A: Bone tissue pathological slices in blank group. Chondrocytes were orderly. The bone trabeculas inside femoral heads were regular, compact and orderly with osteoblasts and little osteoclasts on the side. There were various eumorphism hematopoietic cells and little adipocytes in cavum medullare without cell fusion. B: Bone tissue pathological slices in treatment group. Chondrocytes were relatively orderly. Bone trabeculas inside femoral heads were relatively orderly with osteoblasts and osteoclasts at the side. There were many various hematopoietic cells in cavum medullare. Adipocytes were slightly hyperplastic and hypertrophic without large fat droplet and cell fusion. C: Bone tissue pathological slices in model group. Chondrocytes was hypertrophic. There were sparse, atrophic and incompletely separated bone trabeculas, some necrotic osteocytes inside femoral heads and many osteoclasts. There were obviously hypertrophic and hyperplastic adipocytes in cavum medullare and decreased hematopoietic cells.

Bone mineral density detection

Bone densitometer was applied to detect the bone density of animals in three groups. The results showed that there were significantly statistical differences of rabbits' bone density in three groups (F=14.007, P=2.04×10^{-5}). The results of pairwise comparison showed that the rabbits' bone density in treatment group were significant higher than the model group (P=1.26×10^{-5}<0.05), and there was no obvious difference between treatment group and blank group (P=0.062>0.05). Thus, epimedium treating SONFH was conducive to the increase of bone mineral density (Table 1).

Histopathological observation

The results of femoral histopathological slices of white rabbits in three groups were showed in Table 2. In blank group, the rabbits' femoral model group (P=1.21×10^{-4}<0.05), and there was no significant difference between treatment group and blank group (P=0.125>0.05).
head cartilages had smooth surfaces, rich blood vessels under cartilage, orderly chondrocytes; the bone trabecula in femoral heads were regular, compact and orderly with osteoblasts and little osteoclasts on the side; there were various eumorphism hematopoietic cells and little adipocytes in cavum medullare without cell fusion (Figure 2A). The results in model group (Figure 2C) revealed that part of the chondrocytes was hypertrophic and irregularly arranged and subchondral bone trabeculas were separated and fractured; there were sparse, atrophic and incompletely separated bone trabeculas and some necrotic osteocytes inside femoral heads which showed significant differences with blank group; there were few osteoblasts and many osteoclasts around bone trabeculas; there were obviously hypertrophic and hyperplastic adipocytes in cavum medullare and decreased hematopoietic cells; femoral head cartilages had slightly rough surfaces. In treatment group, femoral head cartilages had slightly smooth surfaces and relatively orderly chondrocytes; bone trabeculas inside femoral heads had normal and relatively orderly osteocytes with osteoblasts and osteoclasts on the side which showed significant differences with model group; the amount of various hematopoietic cells in cavum medullare was more than model group; adipocytes were slightly hyperplastic and hypertrophic without large fat droplet and cell fusion; intramedullary fat area was obviously reduced than model group (Figure 2B).

Discussion

SONFH has many pathological factors including lipid metabolism disorder, fat embolism, intravascular coagulation, inhibition of angiogenesis and apoptosis [11]. Related risk factors include age, long-term treatment, increasing daily dose and total dosage, the common fracture, some inflammatory diseases and immune-related diseases [12]. The reasons of SONFH are that large doses of hormone inhibit the function of osteoblast, reduce the amount of osteoblast, and increase the activity and function of osteoclast, further cause osteoporosis and osteonecrosis of the femoral head [13]. Icarin, the relative component of epimedium, could significantly reduce the activity of blood platelet, decrease the thrombosis in vitro caused by platelet aggregation, improve femoral microcirculation, further improve the blood supply of femoral head [14]. And icariin could reduce the development of osteoclast, improve the proliferation, differentiation and maturation of osteoblast in vitro, enhance bone mineral density, improve the hardness of femoral head, and speed up the repairing of femoral head [15]. The prevention and treatment of collapse caused by hormone-induced osteoporosis plays a role in the prevention and treatment of SONFH. In this research, after the rabbits in treatment group were given epimedium decoction by gavage, we detected the hemorheology indexes and bone mineral density. The whole blood high shear rate (200 s⁻¹), low shear rate (5 s⁻¹), plasma viscosity and hematocrit in treatment group were decreased, which had significant differences with model group (P<0.05). The data indicated that large dose of epimedium decoction could increase blood flow velocity and blood flow volume to achieve effective perfusion of tissue through the decrease of whole blood viscosity, the improvement of hemorheology and the reduction of systemic vascular resistance. In bone mineral density, the treatment group was higher than model group, which had a significant difference (P<0.05); and there was no obviously difference between treatment group and blank group (P>0.05). The results of pathological section showed that the sections of treatment group had osteoblast and part of osteoclast, and sections of model group had little osteoblast and many osteoclast. There were significant difference between treatment group and model group. It indicated that epimedium could reduce osteoclast, increase osteoblast, enhance bone mineral density and promote the regeneration and repair of skeleton. Another research revealed that epimedium could prevent SONFH by blocking hormone-induced abnormal gene expression [16]. Thus, for patients who must take hormone, simultaneously treated with epimedium could early prevent the development of osteonecrosis of the femoral head.

In conclusion, with further development of researches, epimedium decoction could improve hemorheology and blood supply to bone tissue, and provide good nutrition supply and the internal environment for the prevention and improvement of SONFH. Epimedium decoction could promote the production of osteoblast and inhibit the development of osteoclast in bone tissue, which made bone tissue better regenerate. And epimedium decoction increased the
bone mineral density, simultaneously speeded up the repair of bone tissue.

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Disclosure of conflict of interest

None.

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