

Original Article

Effect of 11 β -HSD 1 inhibitor on bone microstructure and bone density in rats with femoral head necrosis

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Abstract

Objectives: To investigate the effect of 11 β -hydroxysteroid dehydrogenase (11 β -HSD1) inhibitor on bone microstructure and bone density in rats with femoral head necrosis. **Methods:** Eighty Sprague-Dawley (SD) rats were selected and randomly divided into two groups. One group was selected for femoral head necrosis modeling. Then the modeled rats were randomly divided into two groups, one group was injected with 11 β -HSD1 inhibitor as the treatment group, and the other group was used as the model. The unmodeled rats were also randomly divided into two groups, one group was injected with 11 β -HSD1 inhibitor as the control group, and the other group was taken as the normal group. The bone microstructure and femoral bone density of 4 groups of rats were observed. **Results:** There were no significant differences in bone microstructure and bone density between the treatment group and the model group before injection ($P > 0.050$), but they were significantly improved after injection ($P < 0.001$). There was no significant difference in superoxide dismutase (SOD) and malondialdehyde (MDA) between the control group and the normal group ($P > 0.050$). SOD increased significantly, and MDA decreased significantly after injection in the treatment group ($P < 0.001$). **Conclusions:** 11 β -HSD1 inhibitor can effectively improve the bone microstructure of femoral head necrosis rats and increase bone density, which can be used as a new scheme for the treatment of femoral head necrosis in the future.

Keywords: 11 β -HSD 1 Inhibitor, Bone Density, Bone Microstructure, MDA, SOD

Introduction

Osteonecrosis of femoral head (ONFH) refers to ischemia, necrosis, and collapse of femoral head due to various reasons that destroy the blood supply of the femoral head, which often causes severe hip joint dysfunction and is a prevalent disease in clinical practice at present¹. Osteonecrosis of femoral head is mostly seen in middle-aged and elderly people. According to statistics, the global incidence rate

has reached 28.91/100000². In recent years, more and more studies have found that the incidence rate of femoral head necrosis is increasing year by year, and the age of the affected population is gradually getting younger³. Femoral head necrosis has a great impact on the hip joint of patients. Serious cases will cause limitation of hip joint flexion, extension, abduction and squatting and other activities, seriously affecting the daily life of patients⁴. At present, the treatment of femoral head necrosis is mainly based on the patient's etiology, youthfulness, lesion degree and lesion site. Surgery is usually the main method⁵, including core decompression, core decompression plus vascular bundle implantation, bone transplantation, femoral head repair and reconstruction, artificial joint replacement, etc.⁶. At present, all kinds of treatment methods have different advantages and disadvantages, but the consistent feature is that they have wide limitations and are more expensive⁷. Therefore, if an effective conservative treatment scheme can be found, there will be a great breakthrough in the clinical treatment

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of femoral head necrosis. Therefore, in recent years, researchers at home and abroad have continuously devoted themselves to exploring and finding possible therapeutic targets for femoral head necrosis. With the deepening of research, more and more studies show that glucocorticoid drugs have an important effect on femoral head necrosis⁸⁻¹⁰. Glucocorticoids play an important role in maintaining bone resorption and bone formation¹¹. The key metabolic enzyme of endogenous glucocorticoids is 11 β -hydroxysteroid dehydrogenase (11 β -HSD1), which is also a pre-receptor regulator of glucocorticoids in tissue level. It can not only catalyze the decrease of cortisol biological activity, but also cause the decrease of glucocorticoid level and affect the stability of bone metabolism¹¹⁻¹³. We suspect that the treatment of femoral head necrosis with 11 β -HSD1 inhibitor can achieve better results, but there is still a lack of relevant research support at home and abroad. Therefore, this experiment established a rat model of femoral head necrosis and applied 11 β -HSD1 inhibitor for treatment, observed the femoral condition of the rat, confirmed the application value of 11 β -HSD1, and improved a new treatment idea for clinic.

Materials and methods

Rat data

Eighty Sprague-Dawley (SD) rats of clean grade were selected as experimental subjects and purchased from Beijing Vitalriver experimental animal technology co., Ltd., with the certificate number of SCXK (Beijing) 2016-0011. The rats were half male and half female, weighed (210 \pm 20) g, were fed in a cage (five rats in a cage) with normal food and light, and kept in an environment of (29 \pm 2) $^{\circ}$ C. This study has been approved by the Animal Ethics Committee of our hospital.

Method

Forty of the total 80 rats were selected by random number table method for femoral head necrosis modeling. The modeling method was based on the research of Wang et al.¹⁴. Prednisolone acetate (24.5 mg/kg) and sodium patulin (140,000 U/rat) were injected intraperitoneally twice per week for 4 weeks. X-ray examination of the bone under articular cartilage in the weight-bearing area on the anterior side of the femoral head showed an arc-shaped transparent band with reduced density. Isotope bone scan or ECT indicated that the femoral head region has a radioactive defect area, and the modeling is determined to be successful. The successfully modeled and unmodeled rats were randomly divided into 2 groups, 20 rats in each group. One group was selected from the modeled and unmodeled rats to treat with 11 β -HSD1 inhibitor (purchased from Beijing Biolab Technology Co., Ltd., M05569-UVE, 8 g/kg) twice per week. The modeled rats were taken as the treatment group and the unmodeled rats as the control group. The other two groups of rats were injected with the same dose of normal saline. The modeled rats were taken as the model

group and the unmodeled rats as the normal group. The 4 groups were injected continuously for 4 weeks. Five rats were randomly selected from each group before injection of 11 β -HSD1 inhibitor (TO), one week after injection (T1), two weeks after injection (T2), and four weeks after injection (T3) after modeling. They were anesthetized with intraperitoneal injection of 10% of chloral hydrate at 350 mg/kg, and then were killed by breaking the neck. Bilateral femoral heads were removed under aseptic conditions. The femoral bone density was scanned by x-ray absorptiometry, and the 11 β -HSD1 level in femoral heads was detected by western blot. In addition, venous blood of rats was collected, and the levels of transforming growth factor- β 1 (TGF- β 1) (Shanghai Gefan Biotechnology Co., Ltd., PEO51) and osteocalcin (BGP) (purchased from Shanghai Guandao Biotechnology Co., Ltd., GD-EOO2274444) in serum were detected by ELISA. Tissue homogenate was prepared from rat brain hippocampal tissue, and superoxide dismutase (SOD) and malondialdehyde (MDA) in hippocampus were detected by ELISA. The kits were purchased from Shanghai Jingkang Biological Engineering Co., Ltd., JK-(a)-5232, JK-(a)-5002.

Observation index

The levels of 11 β -HSD1, femoral bone density, trabecular thickness, trabecular number, trabecular resolution, TGF- β 1, BGP, SOD and MDA in the 4 groups of rats were observed.

Statistical method

SPSS24.0 software (Beijing Strong-vinda Information Technology Co., Ltd.) was used to calculate all experimental results. Graphpad8 (Shenzhen Softhead Software Technology Co., Ltd.) was used to visualize all graphs and the results were checked twice. Measurement data such as BGP and SOD levels were expressed in the form of (mean \pm standard deviation). One-way ANOVA and LSD back testing were used for comparison among groups. Repeated measurement analysis of variance and bonferroni back testing were used for the comparison among multiple time points. The P value less than 0.050 was regarded as statistical significance.

Result

Modeling results and treatment results

Among the 40 modeling rats, 39 were successfully modeled, with a modeling success rate of 97.50%. Therefore, there are 20 in the treatment group, 19 in the model group, 20 in the control group and 20 in the normal group. There was no rat death during the injection experimental treatment, so the treatment group, control group and normal group executed 5 rats each time, the model group executed 5 rats at TO, T1 and T2, executed 4 rats at T3.

Comparison of 11 β -HSD1 levels

At TO, there was no significant difference in 11 β -HSD1 between the control group and the normal group (P>0.050),

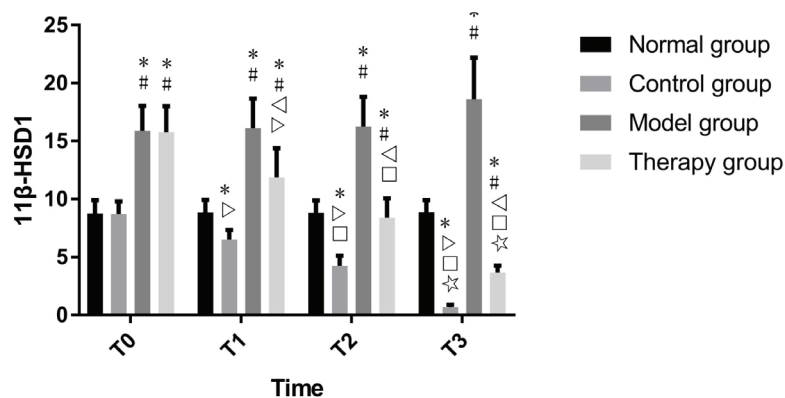


Figure 1. Comparison of 11 β -HSD1 levels in four groups of rats. * represents the level of 11 β -HSD1 compared with the normal group at the same time, $P < 0.001$; # represents the level of 11 β -HSD1 compared with the control group at the same time, $P < 0.001$; Δ represents the level of 11 β -HSD1 compared with the model group at the same time, $P < 0.001$; ∇ represents the level of 11 β -HSD1 compared with the same group at T0, $P < 0.001$; \square represents the level of 11 β -HSD1 compared with the same group at T1, $P < 0.001$; \star represents the level of 11 β -HSD1 compared with the same group at T2, $P < 0.001$.

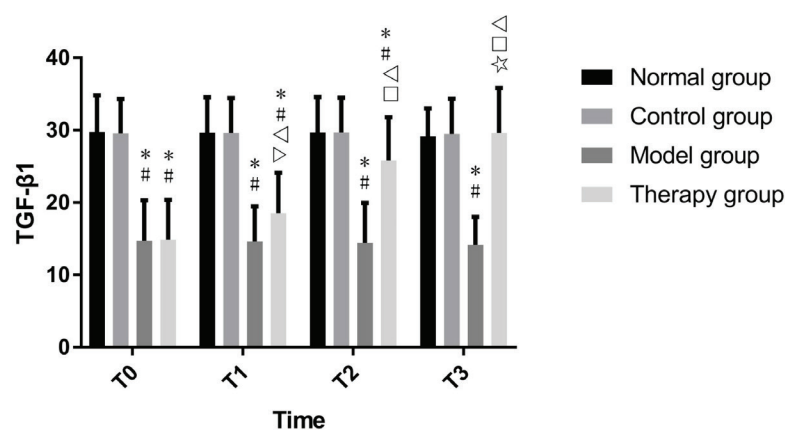


Figure 2. Comparison of TGF- β 1 levels in four groups of rats. * represents the level of TGF- β 1 compared with normal group at the same time, $P < 0.001$; # represents the level of TGF- β 1 compared with the control group at the same time, $P < 0.001$; Δ represents the level of TGF- β 1 compared with the model group at the same time, $P < 0.001$; ∇ represents the level of TGF- β 1 compared with the same group at T0, $P < 0.001$; \square represents the level of TGF- β 1 compared with the same group at T1, $P < 0.001$; \star represents the level of TGF- β 1 compared with the same group at T2, $P < 0.001$.

and there was no significant difference between the model group and the treatment group ($p > 0.050$). The control group and the normal group were significantly lower than the model group and the treatment group ($p < 0.001$). At T1 and T3, 11 β -HSD1 was the highest in the model group, significantly higher in the treatment group than in the normal group, and lowest in the control group ($P < 0.001$). At T2, there was no significant difference between the treatment group and the normal group ($p > 0.050$), 11 β -HSD1 in the two groups was significantly lower than the model group and higher than the control group ($p < 0.001$). There was

no significant difference in 11 β -HSD1 between the normal group and the model group at each time point ($P > 0.050$), while the control group and the treatment group were the highest at T0, began to decrease at T1, and was the lowest T3 ($p < 0.050$) (Figure 1).

Comparison of TGF- β 1 levels

At T0, there was no significant difference in TGF- β 1 between the control group and the normal group ($p > 0.050$), and there was no significant difference between the model group and the treatment group ($p > 0.050$). The control group

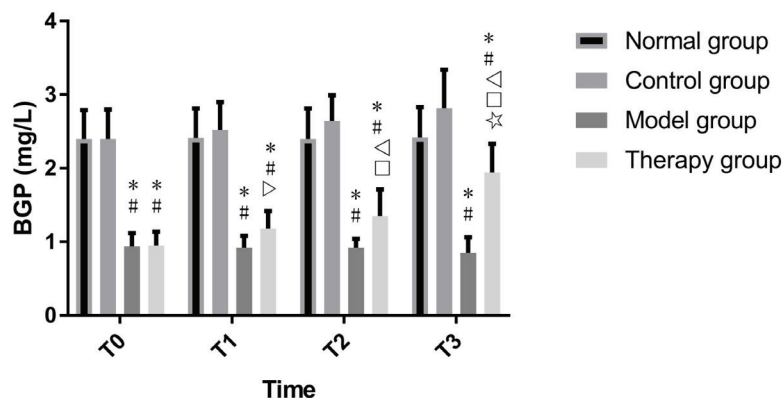


Figure 3. Comparison of BGP levels in four groups of rats. * represents BGP level compared with normal group at the same time, $P < 0.001$; # represents the BGP level compared with the control group at the same time, $P < 0.001$; Δ represents the BGP level compared with the model group at the same time, $P < 0.001$; ∇ represents BGP level compared with the same group at T₀, $P < 0.001$; \square represents BGP level compared with the same group at T₁, $P < 0.001$; \star represents BGP level compared with the same group at T₂, $P < 0.001$.

and the normal group were significantly higher than the treatment group and the model group ($p < 0.001$). At T₁ and T₂, there was no significant difference in TGF- β 1 between the control group and the normal group ($p > 0.050$), TGF- β 1 of the two groups was also significantly higher than that of the treatment group and the model group ($p < 0.001$), while the model group was significantly lower than that of the treatment group ($p < 0.001$). At T₃, there was no significant difference in TGF- β among normal group, control group and treatment group ($p > 0.050$), TGF- β in the three groups was significantly higher than that of model group ($p < 0.001$). There was no significant difference in TGF- β 1 between control group, normal group and model group at each time point ($p > 0.050$). TGF- β 1 in treatment group was lowest at T₀, began to increase at T₁, and was highest at T₃ ($p < 0.001$) (Figure 2).

Comparison of BGP levels

At T₀ and T₁, there was no significant difference in BGP between the control group and the normal group ($p > 0.050$), and there was no significant difference between the model group and the treatment group ($p > 0.050$). The control group and the normal group were significantly higher than the model group and the treatment group ($p < 0.001$). At T₂ and T₃, there was no significant difference in BGP between the control group and the normal group ($p > 0.050$), BGP of the two groups was significantly higher than that of the treatment group, and the model group was the lowest among the four groups ($p < 0.001$). There was no significant difference in BGP among normal group, control group and model group at each time point ($p > 0.050$). BGP in treatment group was lowest at T₀, began to increase at T₁, and was highest at T₃ ($p < 0.001$) (Figure 3).

Comparison of bone density

At T₀ and T₁, there was no significant difference in bone density between the control group and the normal group ($p > 0.050$), and there was no significant difference between the model group and the treatment group ($p > 0.050$). The control group and the normal group were significantly higher than the model group and the treatment group ($P < 0.001$). At T₂, there was no significant difference in bone density between the control group and the normal group ($P > 0.050$), the bone density of the two groups was significantly higher than that of the model group and the treatment group. The bone density of the treatment group was significantly higher than that of the model group ($P < 0.001$). At T₃, there was no significant difference in bone density between the normal group and the treatment group ($P > 0.050$), the bone density of the two groups was significantly higher than that of the model group and lower than that of the control group ($P < 0.001$). There was no significant difference in bone density between normal group and model group at each time point ($P > 0.050$), while there was no significant difference in bone density at T₀, T₁ and T₂ in control group ($P > 0.050$), and the bone density at the three-time points was lower than that at T₄. There was no significant difference in bone density at T₀ and T₁ in the treatment group ($P > 0.050$), but it began to increase significantly at T₂ and reached the highest at T₃ ($P < 0.001$) (Figure 4).

Comparison of bone microstructure

At T₀, T₁, and T₂, there was no significant difference in trabecular thickness, trabecular quantity, and trabecular separation between the control group and the normal group ($P > 0.050$). The thickness and quantity of the control group and the normal group were significantly higher than those of the model group and the treatment group, while the

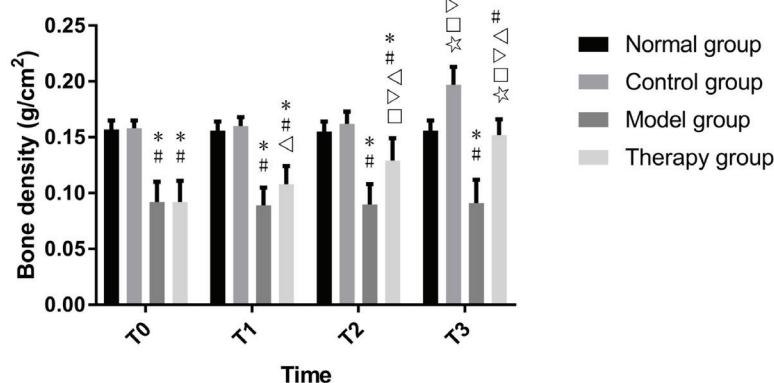


Figure 4. Comparison of bone mineral density of femur in four groups of rats. * represents bone mineral density compared with the normal group at the same time, $P < 0.001$; # represents bone mineral density compared with the control group at the same time, $P < 0.001$; Δ represents bone mineral density compared with the model group at the same time, $P < 0.001$; ∇ represents the bone mineral density compared with the same group at T0, $P < 0.001$; \square represents the bone mineral density compared with the same group at T1, $P < 0.001$; \star represents the bone mineral density compared with the same group at T2, $P < 0.001$.

separation was lower than that of the model group and the treatment group ($P < 0.001$). At T3, there was no significant difference between the treatment group and the normal group in separation and number ($P > 0.050$), the separation and number of the two groups were significantly higher than that of the model group and lower than that of the control group ($P < 0.001$). However, the thickness in the control group was the highest among the 4 groups, the normal group and the treatment group were following, and the model group was the lowest ($P < 0.001$). There was no significant difference in thickness, number and separation among the normal group, the control group, and the model group at each time point ($P > 0.050$), there was no significant difference in thickness of the treatment group at T0, T1 and T2 ($P > 0.050$), the thickness at the three-time points was significantly lower than that at T3 ($P < 0.001$). However, the number was the lowest at T0, increased at T1, and was the highest at T3 ($P < 0.001$). The degree of separation was the highest at T0, decreased at T1, and was the lowest at T4 ($P < 0.001$) (Figure 5).

Comparison of SOD and MDA

At T0 and T1, there was no significant difference in SOD and MDA between the control group and the normal group ($P > 0.050$), there was no significant difference between the model group and the treatment group ($P > 0.050$). SOD in the control group and the normal group was significantly higher than that in the model group and the treatment group, while MDA was significantly lower than that in the model group and the treatment group ($P < 0.001$). There was no significant difference in SOD and MDA between the control group and the normal group at T2 ($P > 0.050$). SOD in the two groups was higher than that in the other two groups and MDA was lower than that in the other two groups ($P < 0.001$). SOD in the treatment group was significantly higher than that in

the model group at T2 and T3, while MDA was lower than that in the model group ($P < 0.001$). At T3, SOD and MDA in the treatment group were not significantly different from those in the normal group and the control group ($P > 0.050$). SOD was significantly higher than that in the model group, and MDA was significantly lower than that in the model group ($P < 0.050$). There was no significant difference in SOD and MDA among the control group, normal group, and model group at each time point ($P > 0.050$). There was no significant difference in SOD at T0 and T1 in the treatment group ($P > 0.050$). SOD began to increase at T2 and reached the highest at T3 ($P < 0.001$). However, MDA was the highest at T0, decreased at T1, and was the lowest at T3 ($P < 0.001$) (Figure 6).

Discussion

Osteonecrosis of the femoral head is one of the common orthopedic diseases in clinical practice. With the deepening of research, the current pathogenesis has reached a consensus at home and abroad. Clinically, chronic alcoholism, long-term use of antibiotics caused skeletal variation, rheumatism, joint pain, fracture and other orthopaedic diseases deterioration are the critical causes of femoral head necrosis¹⁵⁻¹⁷. At present, surgery is usually recommended as the first choice for the treatment of femoral head necrosis in clinical practice. However, with the application of various surgical procedures, its shortcomings are gradually exposed, and the traumatic stress caused by surgery is more likely to cause the occurrence of other complicated diseases of patients^{18,19}. Therefore, finding an effective conservative treatment scheme is a hot and challenging point for clinical research^{20,21}. In this experiment, the effect of 11 β -HSD1 inhibitor on bone microstructure and bone mineral density

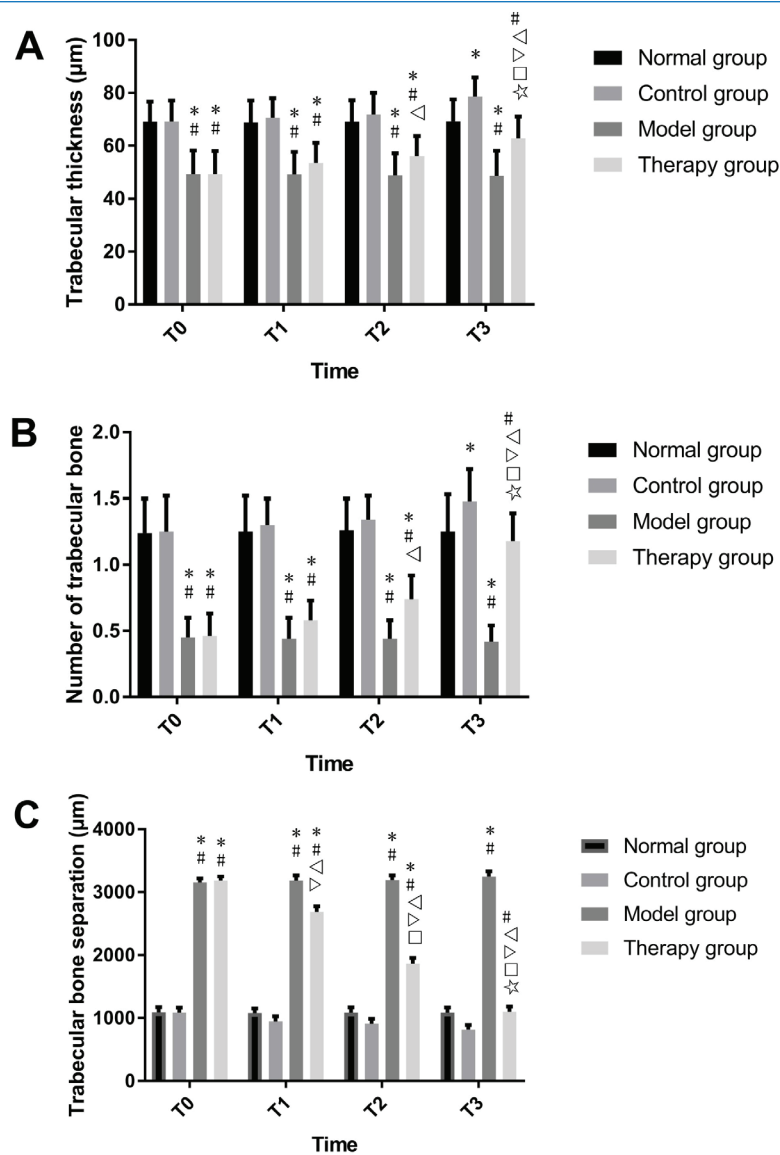


Figure 5. Comparison of bone microstructure of four groups of rats. **A)** comparison of trabecular bone thickness in 4 groups of rats; **B)** comparison of trabecular bone number in 4 groups of rats; **C)** comparison of trabecular bone separation in 4 groups of rats. * represents that compared with normal group at the same time, $P < 0.001$; # represents that compared with the control group at the same time, $P < 0.001$; Δ represents that compared with the model group at the same time, $P < 0.001$; ∇ represents that compared with the same group at T0, $P < 0.001$; \square represents that compared with the same group at T1, $P < 0.001$; \star represents that compared with the same group at T2, $P < 0.001$.

of femoral head necrosis rats was analyzed to prove the feasibility of 11 β -HSD1 inhibitor for the treatment of femoral head necrosis and its clinical significance.

The results of this experiment show that the 11 β -HSD1 level in the treatment group and the control group treated with 11 β -HSD1 inhibitor is significantly lower than that in the model group and the normal group, while the 11 β -HSD1 level in the model group is significantly higher than that in the normal group and the control group, suggesting that 11 β -HSD1 may be involved in the occurrence and development

of femoral head necrosis. 11 β -HSD1 belongs to a short peptide chain ethanol oxidoreductase family, is located in human chromosome 1, and has the structural characteristics of 2 glycosylation sites and 6 exons²². Previous studies have proved that 11 β -HSD1 is closely related to abnormal bone metabolism caused by glucocorticoid. 11 β -HSD1 can enhance the half-life of cortisol, cause hormone activation effect on bone tissue, and destroy the normal development and metabolism of bone trabecula in micro-environment²³. However, through the use of 11 β -HSD1 inhibitor, the

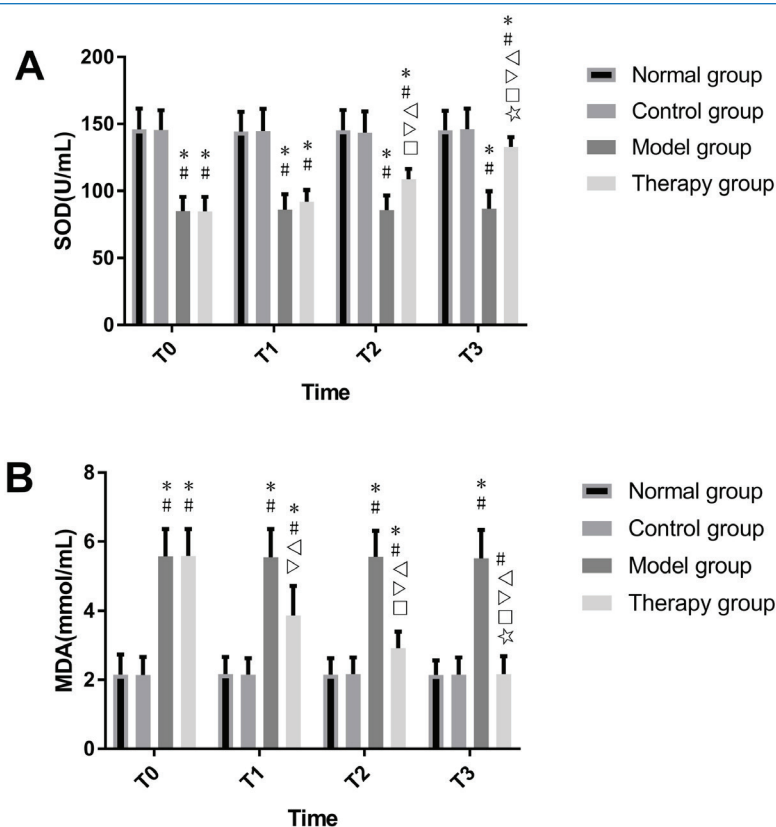


Figure 6. Comparison of SOD and MDA in four groups of rats. A) comparison of SOD in 4 groups of rats; B) comparison of MDA in 4 groups of rats. * represents that compared with normal group at the same time, $P < 0.001$; # represents that compared with the control group at the same time, $P < 0.001$; Δ represents that compared with the model group at the same time, $P < 0.001$; ∇ represents that compared with the same group at T0, $P < 0.001$; \square represents that compared with the same group at T1, $P < 0.001$; \star represents that compared with the same group at T2, $P < 0.001$.

destruction of 11 β -HSD1 on bone microstructure and bone metabolism ability is reduced, which is of great significance for the promotion of bone state in the internal environment. To verify this, we tested the femoral bone density and bone microstructure of rats in each group and found that the bone density and bone microstructure of rats in the treatment group using 11 β -HSD1 inhibitor were gradually improved during the injection process. After the injection, the femoral bone density, bone trabecula separation degree, and bone trabecula number had no significant difference with those of rats in the normal group, suggesting that the therapeutic effect of 11 β -HSD1 inhibitor on femoral head necrosis is satisfactory, and it can be used as a clinical treatment scheme for femoral head necrosis in the future.

Furthermore, the level of TGF- β 1 in rats of each group was detected. The results showed that there was no significant difference between the control group and the normal group, while the TGF- β 1 in the treatment group was significantly higher than that in the model group. TGF- β 1, as a multifunctional cytokine, can transform the phenotype of normal fibroblasts, affecting cell growth, differentiation

and synthesis of extracellular matrix²⁴. However, TGF- β 1 is also of great significance in the process of bone formation, fracture healing and repair²⁵. In the experimental results, TGF- β 1 in the model group and the treatment group increased significantly. We speculated that TGF- β 1 enhanced the differentiation and proliferation of osteoblasts and stimulated the formation of new bone and blood vessels during the injection of 11 β -HSD1 inhibitor. The relationship may be that 11 β -HSD1 regulates the activation of bone cells through TGF- β 1 signaling pathway, but this requires further experiments to verify our hypothesis.

However, through the detection of SOD and MDA in each group of rats, it can be seen that there is no significant difference in SOD and MDA between the normal group and the control group. SOD and MDA in the model group are significantly reduced, while those in the treatment group are gradually increased with the injection of 11 β -HSD1 inhibitor. MDA, as an excellent indicator of lipid peroxidation and oxygen free radicals, is formed by the destruction of DNA, tissue cells and biomembranes caused by excessive reactive oxygen species of oxygen free radicals in an unbalanced state²⁶.

SOD is a metal enzyme substance widely existing in animals, plants and microorganisms, and is a natural scavenger of O₂. However, the results of this experiment show that the injection of 11 β -HSD1 inhibitor will not affect MDA and SOD in normal rats, further confirming the feasibility of 11 β -HSD1 inhibitor in clinical application in the future, and the condition of rats in the treatment group will also gradually be improved during the treatment process, suggesting that 11 β -HSD1 inhibitor also has positive effects on antioxidation and free radical scavenging, and it also has better protective effects on the neurological function of elderly with high incidence of femoral head necrosis.

In this experiment, the effects of 11 β -HSD1 inhibitor on bone microstructure and bone mineral density of femoral head necrosis rats were studied, and there were still deficiencies due to the limited experimental conditions. For example, the mechanism of 11 β -HSD1 on femoral head necrosis is still at the stage of speculation due to a lack of relevant references. It is hoped that some professional scholars can carry out experiments to confirm it according to our views in the future. However, due to the differences between animal models and human beings, we need more detailed human experiments to further determine the availability of 11 β -HSD1 inhibitor for femoral head necrosis. In the future, we will conduct more detailed and in-depth experimental analysis on the application of 11 β -HSD1 inhibitor to provide more comprehensive reference guide for clinical practice.

To sum up, 11 β -HSD1 inhibitor can effectively improve the bone microstructure and bone mineral density of femoral head necrosis rats and can be used as a new clinical treatment for femoral head necrosis in the future.

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Authors' contributions

FL wrote the manuscript. CZ and MG performed Western blot. LX and GW were responsible for ELISA. JZ and JY contributed to observation indexes analysis. The final version was read and adopted by all the authors. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The 2nd Affiliated Hospital of Harbin Medical University.

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