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Study on the Effect of Psoralen on Osteoporosis in Ovariectomized Rats

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Abstract

Objective: The bilateral ovaries of rats were removed to cause hypoestrogen to establish a rat model of osteoporosis. The rats were administered with different doses of Psoralen gavage to observe its effect on improving osteoporosis in rats after ovariectomy. The possible mechanism was explored.

Methods: In vivo experiment: the rat bilateral ovaries were removed to establish a model. After 12 weeks of drug intervention, specimens were obtained to compare the weight of the uterus, and the bone transformation indicators were measured by ELISA. In vitro experiment: Osteoblast precursor cells MC3T3-E1 were cultured, then added with osteogenic induction solution to induce differentiation. The cells were intervened with different concentrations of Psoralen to induce differentiation and then cultured for 21 days. The number of calcium nodules was compared through Alizarin Red staining. Western blotting was used to detect the expressions of ERK/Wnt/β-catenin signaling pathway-related proteins in total cell protein.

Results: 12 weeks after the removal of bilateral ovaries, ovariectomized rats developed osteoporosis. Psoralen intervention can reduce bone loss in ovariectomized rats, enhance the viability of MC3T3-E1 cells, and promote their osteogenic differentiation. After different concentrations of Psoralen intervention, the expressions of ERK1/2, β-catenin, BMP-2, p-ERK1/2 proteins increased, while the expressions of LPL, GSK-3β and PPAR-γ proteins decreased, indicating that Psoralen improved bone quality. The mechanism of porosity improvement may be related to Psoralen regulating ERK/Wnt/β-catenin signaling pathway-related proteins to promote osteoblast differentiation.

Conclusion: Simple hypoestrogen can cause osteoporosis in rats, and the intervention of Psoralen can protect the bones. The anti-osteoporosis mechanism may be related to its regulation of ERK/Wnt/β-catenin signaling pathway to promote the formation of osteoblasts.

Keywords: Psoralen; Osteoporosis; ERK/Wnt/β-catenin signaling pathway

Introduction

Psoralen can stop bleeding in the uterus, nose and gums, and relax bronchial smooth muscles. However, it is still unclear whether Psoralen can promote ERK phosphorylation, inhibit the activity of GSK-3P enzyme, activate the activity of the Wnt/β-catenin signaling pathway, and promote the differentiation of osteoblasts. There is no such research in this aspect yet, so this experiment takes an entry point to study the effect of Psoralen on the differentiation mechanism of osteoblasts.

Modern experiments show that Psoralen, an effective monomer component, can promote the proliferation and differentiation and maturation of osteoblasts, inhibit the viability of osteoclasts, and play a dual role in promoting bone formation and inhibiting bone resorption, so it can be used for bone porosity treatment. At present, Psoralen granules and Psoralen injections have been used in small-scale clinical applications, and the curative effect is good, with small side effects, indicating that Psoralen has broad application prospects in the field of orthopedics [1-2].

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Methods.

1. Establishment of osteoporosis model and grouping

Rats undergo surgery to remove both ovaries. In the sham operation group rats, only a small piece of adipose tissue around the ovarian tissue was removed during the operation, and drug

intervention was performed two weeks after the operation. The rats were divided into four groups: A, the sham operation group (Sham), in which the rats were gavage with 1.5 ml physiological saline solution once a day; B, the Control group, in which the rats were gavage with 1.5 ml physiological saline solution once a day; C, the low-dose Psoralen group, in which the rats were given Psoralen 10 mg/Kg once a day; D, the Psoralen medium-dose group, in which the rats were given Psoralen 20 mg/Kg once a day; and E, the Psoralen high-dose group, in which the rats were given Psoralen 40 mg/Kg once a day.

2. Serum bone transformation index detection.

The bone formation and resorption indices were measured, and the ELISA kit was used for the determination.

3. Determination of bone density

The left tibia was obtained. The tibia at about one-third away from the distal end were broken off, then put in 4% paraformaldehyde solution. The tibia was fixed at 4 °C for 48 hours, then scanned with microcomputer tomography technology. Micro-CT was applied as a method of measuring bone density.

4. Cell grouping and CCK-8 method to measure cell viability

MC3T3-E1 cells in good condition were obtained. The absorbance at 450 nm was measured with a CCK-8 microplate reader. Cells are grouped as follows: A, the Control group, with untreated cells; B, the Psoralen low concentration group, in which the cells were treated with10 μM psoralen for 4 h; C, the Psoralen medium concentration group, in which the cells were treated with 20 μM Psoralen for 4 h; and D, the Psoralen high-concentration group, in which the cells were treated with 40 μM Psoralen for 4 h.

5. Osteogenic induction and Alizarin Red staining of MC3T3-E1 cells

MC3T3-E1 cells were inoculated in a culture medium with 10% FBS, and a complete medium containing osteogenic induction culture medium was added. The cells gradually fused. After 21 days of co-cultivation, mineralized nodules were formed and stained with Alizarin Red.

6. WB method to determine the content of each protein

Western blotting was used to detect the expressions of ERK/Wnt/β-catenin signaling pathway-related proteins in total cell protein.

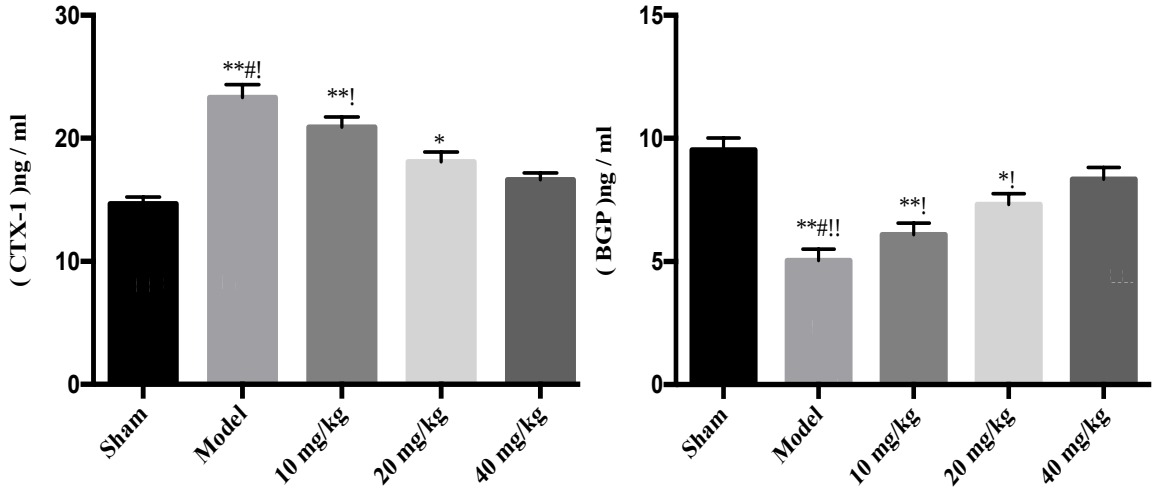
7. Statistical analysis

All statistical analyses were performed using SPSS software (Version 19.0, SPSS Inc, Chicago, USA).

Results.

1. The effect of Psoralen on serum bone transformation markers in rats.

Compared with the sham operation group, the concentration of CTX-I in the serum of the model group increased, while the concentration of BGP decreased. Compared with the model group, after drug administration, CTX-I reduced and the depth of BGP increased, as shown in Figure 1.

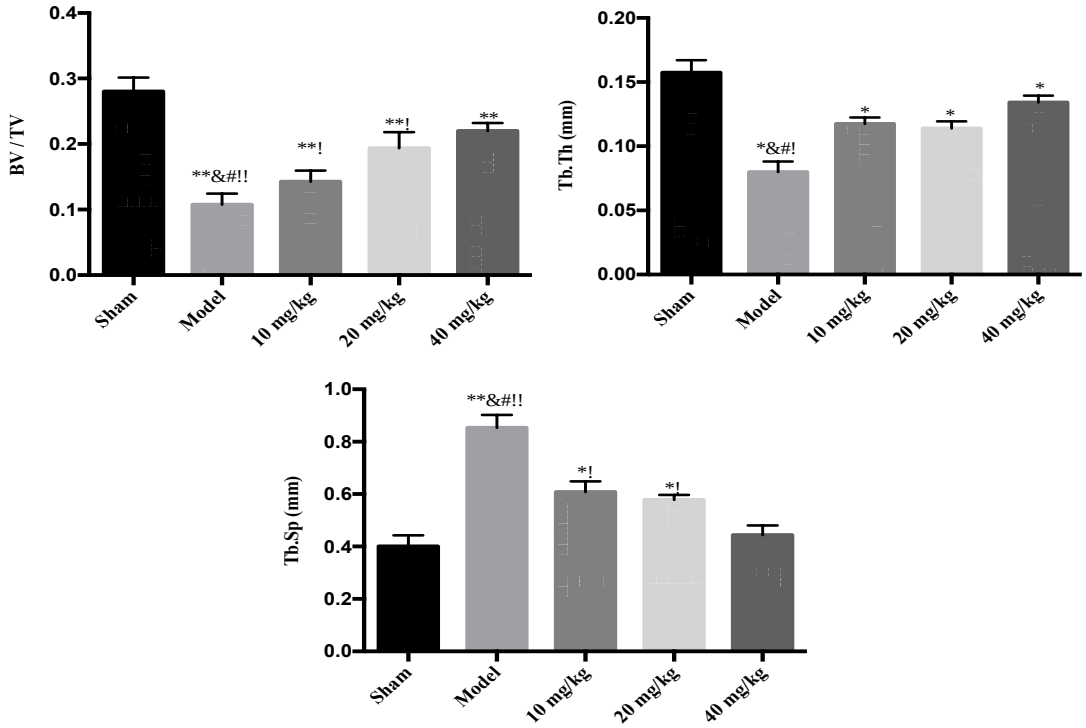


** p < 0.01 vs Sham * p < 0.05 vs Sham # p < 0.05 vs 20 mg/kg ! p < 0.05 vs 40 mg/kg

Figure.1 **The effect of Psoralen on the serum CTX-I and BGP in rats.**

2. The effects of Psoralen on bone mass/total volume ratio, trabecular bone thickness and trabecular bone porosity.

Compared with the sham operation group, in the model group, BV/TV and Tb.Th decreased, and Tb.Sp increased, which was consistent with the pathological changes of osteoporosis. Compared with the model group, the BV/TV, Tb.Th and Tb.Sp of the drug group were increased, and Tb.Sp was decreased, suggesting that drug intervention can reduce the formation of osteoporosis in ovariectomized rats, as shown in Figure 2.



** p < 0.01 vs Sham * p < 0.05 vs Sham & p < 0.05 vs 10 mg/kg
# p < 0.05 vs 20 mg/kg !! p < 0.01 vs 40 mg/kg ! p < 0.05 vs 40 mg/kg

Figure 2. **The effects of Psoralen on bone mass/total volume ratio, bone trabecular thickness and bone trabecular porosity.**

3. The effect of Psoralen on MC3T3-E1 cell viability.

CCK-8 was employed to detect cell viability. After drug intervention, the cell viability of the administration group increased compared with the blank group, as shown in Figure 3.

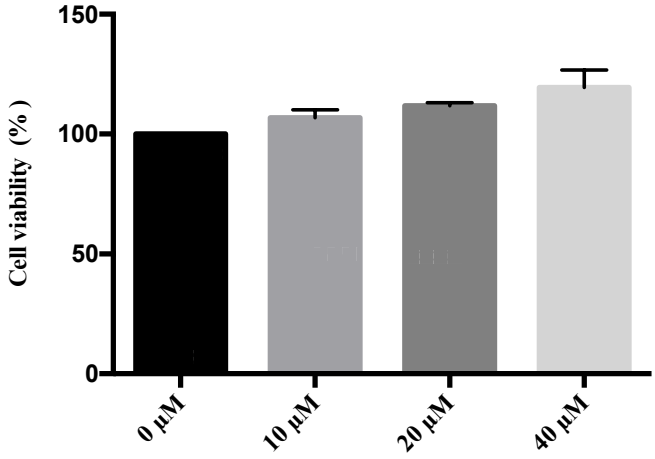


Figure 3. **The effect of Psoralen on the viability of MC3T3-E1 cells**

4. The effect of Psoralen on osteogenic differentiation of MC3T3-E1 cells.

MC3T3-E1 cells were cultured with osteogenic induction medium for 21 days. The formation of mineralized nodules was observed by Alizarin Red staining. Compared with the blank group, osteoblast differentiation and mineralized nodules increased in the drug intervention group, as shown in Figure 4.

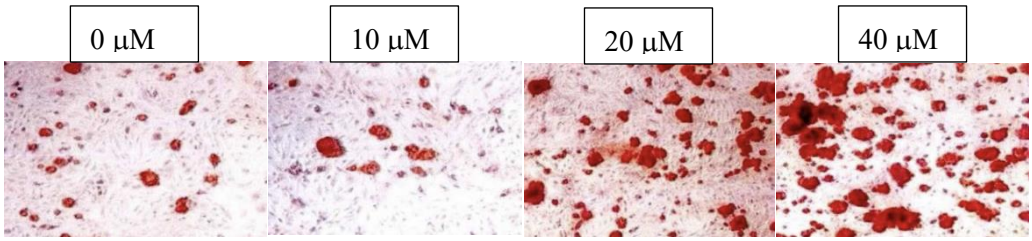


Figure 4. **The effect of Psoralen on the osteogenic differentiation of MC3T3-E1 cells**

5. The effect of Psoralen on ERK signaling pathway in MC3T3-E1 cells.

After 21 days of intervention with Psoralen, the protein expression was measured by Western blotting. Compared with the blank group, the expressions of ERK1/2, p-ERK1/2, β-catenin and BMP-2 proteins increased after intervention with different concentrations of drugs. The expressions of LPL, GSK-3β and PPAR-γ proteins were down-regulated, as shown in Figure 5.

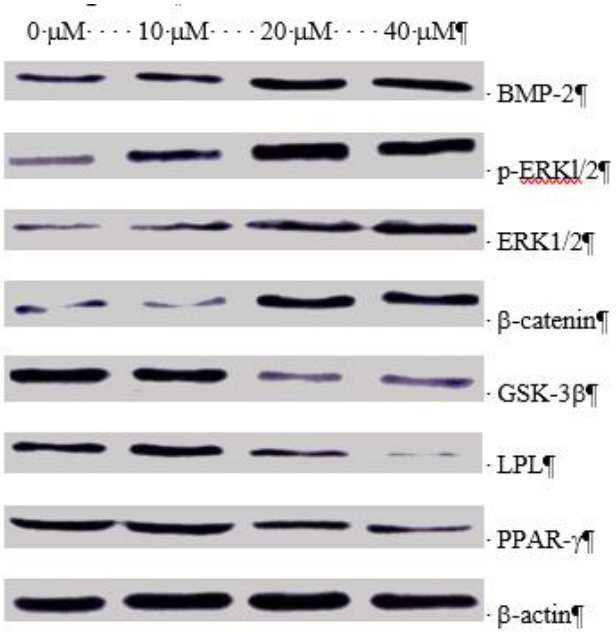


Figure 5. **The effect of Psoralen on various proteins.**

Discussion

In the physiological development of women, perimenopause is a key node, which usually occurs between 40-55 years old. Contrary to menstrual cramps, menopause marks the end of a female's reproductive phase. When a woman reaches the age of 35, the function of the ovaries gradually begins to degenerate, the level of estrogen decreases, and calcium begins to be lost. The transition from a latent reproductive state to a non-reproductive state does not happen suddenly, but in a gradual process, which usually takes several years.

Most studies believe that estrogen has a certain role in promoting osteogenic differentiation. Co-regulatory factors related to estrogen receptors are found in the nucleus of mature osteoclasts. Estrogen can promote osteoblasts to secrete osteoprotegerin and can competitively bind with osteoclast differentiation factor receptors on the surface of osteoclasts, thereby inhibiting the differentiation and maturation of osteoclasts, and playing an important role in maintaining the balance between bone resorption and bone formation in women. The gradual degradation of ovarian function in postmenopausal women causes

a sharp decline in estrogen in the body. The sudden decline in estrogen disrupts the balance between bone resorption and bone formation, resulting in a significant increase in bone resorption and high bone turnover. Under the condition, it is difficult to maintain normal bone mass, leading to a decrease in bone mass and bone strength. Clinical manifestations are as such pain in the back or whole body, shortening of height, hunchback and even fractures [2-8].

The Wnt/β-catenin signaling pathway plays an important regulatory role in cell growth and apoptosis. Human gene research has confirmed Wm signaling as a key mechanism to stimulate osteoblast differentiation and viability. In domestic studies, ELISA was applied to detect the expression of β-catenin in the serum of postmenopausal osteoporosis patients. It is found that β-catenin, as an important conduction factor of the Wnt signaling pathway, is more obviously reduced in postmenopausal osteoporosis patients than in normal postmenopausal women [9-10]. Wnt protein can inhibit the apoptosis of osteoblast precursor cells, thereby promoting osteoblast differentiation. There are many signal transduction proteins that are closely related to the Wnt/β-catenin signaling pathway. Among them, a type of extracellular regulatory protein kinase discovered in the late 1980s can transmit mitogen signals. It is the key to transmit signals from surface receptors to the nucleus.

The ERK signaling pathway is currently well studied, and it is one of the most important pathways for most cytokines and growth factors to regulate cell proliferation [11-12]. The ERK pathway plays an important role in maintaining the balance between bone formation and bone resorption. It promotes the differentiation and formation of bone mesenchymal stem cells into osteoblasts, regulates the expression of osteoprotegerin and nuclear receptor activator, and can inhibit the expression of transcription factor Runx2. Cells can transmit various signal stimuli outside the cell and various mechanical stimuli through the ERK signaling pathway to affect signal transmission inside and outside the cell [13-14].

In the experiment, common MC3T3-E1 cells have been used for research and CCK-8 experiments have confirmed that Psoralen is non-toxic to cells at concentrations of 0 μM, 10 μM, 20 μM and 40 μM. Psoralen can enhance cell viability, and the mechanism is speculated to be related to its antioxidant effect. The intervention of different concentrations of Psoralen in the process of osteogenic differentiation induction of MC3T3-E1 cells with osteogenic induction fluid can increase the number of mineralized nodules. The protein was detected by Western blotting, and it was found that drug intervention can increase the expressions of β-catenin, ERK1/2, and p-ERK1/2 proteins, and attenuate the expression of GSK-3β protein in a dose-dependent manner. The results showed that Psoralen intervention can increase the viability of ERK1/2 signaling pathway, increase the expression of p-ERK1/2, reduce the viability of GSK-3β, and further activate the Wnt/β-catenin signaling pathway to promote osteoblast differentiation. Different concentrations of Psoralen can significantly enhance the viability of MC3T3-E1 cells and promote the differentiation of osteoblasts.

Liposome-generating enzyme is an early classic marker of the differentiation of bone marrow mesenchymal stem cells into adipocytes. Lipoprotein expression increases with adipocyte differentiation. In this study, Psoralen increased the viability of osteoblast precursor cells, promoted the differentiation of osteoblast precursor cells into osteoblasts, and increased the expression of p-ERK1/2 in the process of promoting successful differentiation. Psoralen reduced the expression of PPAR-γ, and weakened the expressions of LPL in MC3T3-E1 cells, which may be one of its anti-osteoporosis mechanisms. However, whether Psoralen only stimulates the Wnt/β-catenin signaling pathway through the ERK signaling pathway to promote osteogenic differentiation and achieve anti-osteoporosis effects requires further research.

The present research clarified the effect of Psoralen on osteoporosis in ovariectomized rats, further studied the pathological basis and pathogenesis of postmenopausal osteoporosis, and simultaneously explored the mechanism of Psoralen improving osteoporosis from different signaling pathways.But,this study didn't clarify the pathogenesis of postmenopausal osteoporosis, neither explained the mechanism by which Psoralen can improve perimenopausal osteoporosis from multiple perspectives.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

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None.

Competing interests

There are no potential conflicts of interest to disclose.

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