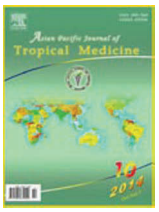
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm



Document heading doi: 10.1016/S1995-7645(14)60140-1

Effects of aspirin on fracture healing in OPF rats

Jin-Song Wei, Rong Zeng*, Si-Yuan Chen, Hao Lin, Shao-Ke Wu, Jin-Chang Zheng

Department of Orthopaedics, Guangdong Medical University Affiliated Hospital, Zhanjiang, Guangdong 524001, China

ARTICLE INFO

Article history:
Received 10 April 2014
Received in revised form 15 May 2014
Accepted 15 July 2014
Available online 20 October 2014

Keywords:
Aspirin
Osteoporosis
Fracture
Healing

ABSTRACT

Objective: To study the effect of aspirin on healing process of osteoporotic fracture (OPF) in rats. **Methods:** A total of 50 female Wistar rats aged 3 months were randomly divided into observation group and control group, castration method was adopted to establish the osteoporosis (OP) model. After artificial preparing fractures on the midpoint of left femur, fixing gram needle intramedullary, OPF modeling was complete. Aspirin lavage of 33 mg once a day was adopted in observation group after modeling, same amount of normal saline was used in the control as placebo. From each group, selected 5 rats at the 2nd, 4th, 8th and 12th week after modeling to measure the bone mineral density (BMD) and histological examination of the fracture callus, radiology observation was conducted at the 8th and 12th week. Left femur biomechanical measurement was taken at the 12th week. **Results:** BMD values of observation group at each time point were significantly higher than that of the control group after modeling ($P<0.05$); Histological observation showed that at the 8th week, the endochondral ossification process of observation group was faster than that of observation group, with fuzzy fracture line in observation group and clear fracture line in observation group; at the 12th week, fracture line disappeared in observation group, fracture line of the control group was fuzzy at the same time; three-point bending load of the left femur in observation group rats was significantly higher than that of control group after 12 weeks ($P<0.05$). **Conclusions:** Aspirin can accelerate the healing of new callus in OPF rats, increase bone density and biomechanics strength, and promote fracture healing of osteoporotic rats.

1. Introduction

As the population aging process Increased, the diseases caused by physiological changes due to aging are increasing[1]. Osteoporosis (OP) is a common systemic bone metabolic diseases in the elderly, causing serious harms to this group of people[2]. Primary pathological manifestations of OP are the decrease of bone mass and the damage of bone microstructure, resulting in an increase in brittleness and a decline in intensity of bones, and easy to progress into Osteoporoticfracture (OPF)[3]. OPF is one of the most serious complications of OP, drawing an extensive attention clinically. OPF often occurs at the hips, vertebral body and wrist, with the highest incidence and mortality at hip fracture[4]. At present, the main drugs used in the treatment of OP have effects of resistanting to re-absorption, promoting bone formation and bone mineralization drugs, etc.; Clinical treatment of OPF mainly include surgery and conservative treatment, combination therapy of surgery with effective treatment target for the primary disease OP after fracture reduction and fixation can reduced complications and mortality, and can significantly increase the rate of fracture healing[5]. Aspirin is a common drug with long history of clinical use, its new pharmacological effects have been found during the process of clinical application[6]. Studies have shown that[7], small doses of aspirin can promote trabecular bone reconstruction in postmenopausal OP rats, improve the three-dimensional structure of trabecular bone, increase bone density and mechanical strength. To observe the effects of aspirin on OPF healing process, the author selected female Wistar rats of 3 months to establish the model of OP, prepared fractures

*Corresponding author: Rong Zeng, M.B., Professor, Chief Physician, Department of Orthopaedics, Guangdong Medical University Affiliated Hospital, Zhanjiang, Guangdong 524001, China.
Tel: 0759-2369111
E-mail: zenrong1964@163.com
Foundation Project: It is supported by Guangdong Science and Technology Projects, grant No. 2010 b031600288.

on the midpoint of the left femur, followed by aspirin lavage treatment to observe the fracture healing, bone mineral density and histological changes at different time points.

2. Materials and methods

2.1. Experimental animals

A total of 50 healthy female Wistar rats of 3 months were selected, which were grade clean, 245-300 g, offered by the experimental animal center of our hospital. Free access to water and food was provided. The condition was as follows: class Ⅱ, raising humidity of 30%-45%, temperature of 20-30 ℃, regular ventilation and lighting. Packing was changed once a day, experiments on animals strictly followed the related provisions in the Regulations for the Administration of Affairs Concerning Experimental Animals.

2.2. Reagent and instrument

Aspirin (Batch number: H32026201, Astrazeneca Pharmaceutical Co., LTD.); 10% chloral hydrate, provided by the National Medicine Group Chemical Reagent Beijing Co., LTD.; DR digital camera (Philip, Germany); Discovey-Wi dual-energy X-ray absorptiometry bone mineral density instrument (HOLOGIC, USA); BI-2000 medical image analysis system (Western Science and Technology Co., LTD. Wuhan); Optical microscope (Leica Company, Germany); 1.2 mm gram needles.

2.3. Model establishment

2.3.1. OP model preparation

Intraperitoneal injection of 10% chloral hydrate of 3 mL/kg was used for anesthesia, bilateral ovaries were resected from the rats′back, then the wound was sealed. In order to prevent infection, 80 000 units of penicillin was injected intraperitoneal continuously for 3 d, 2 times a day; They were provided with free access to water, food and activity. After 8 weeks, BMP was measured using dual energy X-ray absorptiometry testing, and compared with normal rats. BMP of normal rats and experimental rats was (0.260±0.005) g/cm^2 and (0.208±0.008) g/cm^2 respectively, which confirmed the establishment of OP model.

2.3.2. OPF model preparation

After the success of OP model establishment, they were intraperitoneal injected with 10% chloral hydrate anesthesia of 3 ml/kg. Then regular disinfection was performed, and they were incised along the unilateral femoral middle. The layers of tissue was separated, the femur was exposed; Midpoint of the femoral periosteum was cut, femur was sawed and fixed with the gram needles, then the incision was closed. In order to prevent infection, they were intraperitoneal injected with 80 000 units of penicillin for 3 d continuously, 2 times a day. Free access to activities, water and foods were provided to rats.

2.4. Animal groups

A total of 50 female Wistar rats aged 3 months were randomly divided into observation group and control group. After OPF modeling, aspirin lavage of 33 mg once a day was adopted in observation group after modeling, same amount of normal saline was used in the control as placebo.

2.5. Experiment method

2.5.1. Femoral determination of BMP

From each group, 5 rats were selected on the 2nd, 4th, 8th and 12th week after modeling, the gram needles were removed. The bone mineral density (BMD) was measured around 2 .0 mm×1.5 mm of the fracture callus using Discovey-Wi dual-energy X-ray absorptiometry.

2.5.2. X-ray

Chloral hydrate anesthesia was conducted in abdominal cavity of rats on the 8th and 12th week, X-ray radiography examination was used for observing the fracture healing.

2.5.3. Histological observation

After testing the BMD, the lateral femoral of surgery side was intercepted. The middle part of femoral and callus tissue was cut out, and was conventional fixed. They were decalcified with 20% EDTA for 4 weeks, gradient ethanol dehydrated, transparented, embedded with xylene paraffin, sliced in 4 μ μ m section, and stained by HE. The changes of bone tissue at each time point was observed under light microscopy, BI-2000 medical image analysis system was used for calculation of density per unit area of new trabecular bone.

2.5.4. Biomechanical measurement

After 12 weeks of treatment, serum calcium (Ca), phosphorus (P), alkaline phosphatase (ALP) concentration of peripheral blood were tested before the execution of rats. The soft tissue was extracted from the lateral femoral, the needles were removed, covered by saline gauze, and stored at 20 ℃, three point bending load test was made for biomechanical test.

2.6. Statistical methods

SPSS19.0 statistical software was used, t test was used for comparing the difference between groups; $P<0.05$ was considered as statistically significant difference.

3. Results

3.1. BMP value comparison of two groups

BMD values of observation group after treatment of 2, 4, 8 and 12 weeks were significantly higher than that of the control group after modeling ($P<0.05$) (Table 1).

3.2. Fracture healing at 8th and 12th week of treatment

On the 8th week, callus was connected closely at fracture end in observation group, with blurred fracture lines, after 12 weeks, callus density was significantly higher than that at 8 weeks and fracture line disappeared in observation group; at the 8th week, fracture line of control group was clear, became faint after 12 weeks of treatment, fracture callus density was lower than that of observation group (Figure 1).

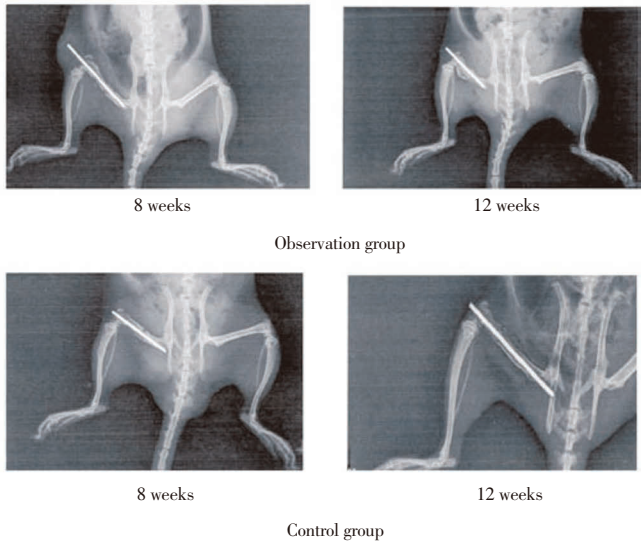


Figure 1. Fracture healing at 8th and 12th week of treatment.

3.3. Histological observation

Three-point bending load of the left femur in observation group rats was significantly higher than that of control group after 12 weeks ($P<0.05$). 2 weeks later, fracture cell mass, fiber callus and cartilage island began to form in observation group, with more cartilage island area; 4 weeks later, a large number of new cartilage cells, osteoclasts and osteoblasts can be observed in the observation group, with a small amount of original trabecular bone, and a few new cartilage cells, original trabecular bone was not observed in the control group, as shown in Figure 2; 8 weeks later, the trabecular bone formated in great quantities in observation group, between which the capillary distribution was rich. In the control group, the trabecular bone formation was less, a large number of mast cartilage cells were still visible, as shown in Figure 2; 12 weeks later, in observation group,coarser trabecular bone displayed in higher density in the same direction. In the control group, finer bone trabecular gap was larger in a disordered orientation (Figure 2).

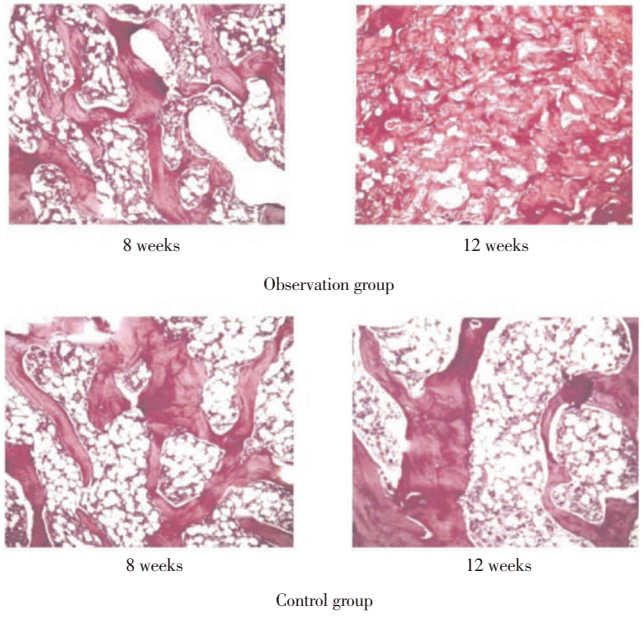


Figure 2. Callus histological observation at different time points (HE, ×200).

Table 1
Femoral BMP value at different time points (g/cm^3)

Groups	2 weeks	4 weeks	8 weeks	12 weeks
Observation group	0.094±0.005*	0.172±0.006*	0.201±0.005*	0.252±0.006*
Control group	0.080±0.006	0.152±0.005	0.182±0.006	0.206±0.008

Note: * compared with controls ($P<0.05$).

Table 2
Serum concentration of Ca, P and ALP between two groups after 12 weeks of treatment.

Groups	Ca (mmol /L)	P (mmol /L)	ALP (U/L)
Observation group	2.60±074*	2.61±0.34*	97.8±9.8*
Control group	3.48±0.20	2.26±0.74	165.0±23.5

Note: * compared with controls ($P<0.05$).

3.4. Serum concentration of Ca, P, ALP and biomechanical measurement results after12 weeks of treatment

Observation group showed a significantly lower levels of serum ALP and Ca, and a significantly higher serum P level after 12 weeks of treatment, compared with control group (P<0.05), as shown in Table 2. Observation group showed a significantly higher three-point bending load test results of (175.9±3.41)N, compared to the control group with (164.9±3.42) N (P<0.05) (Table 2).

4. Discussion

OP refers to bone microstructure degeneration and systemic bone disease characterized by low bone mass, according to statistics, OP rate is as high as 50%-70%in our country older women[8]. With the increase of population life expectancy, and changes in diet and lifestyle, the OP incidence increased year by year[9]. OP patients tend to occur fractures, seriously affect the quality of life. BMD is reduced in OP patients, bone quality significantly reduced, bone load ability is reduced, its own gravity or minor external force can lead to fractures[10-13]. OPF is a serious complication of OP, OP is one of the most serious consequences of OPF, often occurs in the spine and femur bone, but also different from traumatic fracture, most belong to complete fracture, only a few cases are simple intramedullary trabecular micro fractures[15-17]. combination therapy of surgery with effective treatment target for the primary disease OP after fracture reduction and fixation can reduced complications and mortality, and can significantly increase the rate of fracture healing[18]. This study established OP model by ovarian castrated surgery followed by 8 weeks feeding, BMP showed was a significant reduction in the observation group, compared with the normal rats, suggesting that ovarian castrated surgery to build OP model is fast and effective. Preparation of OPF model mainly adopts closed and open types[19]. Preparation methods of closed fracture model causes ild injury of surrounding soft tissue, periosteum complete can be conducive to fracture healing, but this method has difficulties in control, and the model standards are hard to determine[20]. This study adopted open preparation methods for modeling causing relative severe trauma, but similar degree of trauma in this model can ensure the comparability of experimental rat fracture healing[21].

Aspirin clinical has a long time application in a wide range of disease treatment with relatively minor side effects. its new uses are still being discovered constantly. Aspirin can prevent thrombosis, has inhibition to the platelet aggregation, can be used to prevent temporary cardiac and cerebral vascular thrombosis after operation; it can also be used in the treatment of unstable angina, rheumatoid arthritis, ankylosing spondylitis, diseases such as osteoarthritis; and for diabetes, athlete′s foot, gestational hypertension, migraine, senile cataract, gastrointestinal cancer, senile dementia, lower limb varicose ulcer[22]. In recent years, studies have shown that[23], BMD of the old people who take aspirin regularly for a long time is higher than that of the elderly without regular medication of aspirin. Another study based on micro CT analysis found that[24], low doses of aspirin treatment in castrated mice for 3 months showed significantly improved bone trabecular and cortical bone mineral density, suggesting that aspirin may have an impact on rat bone reconstruction process, with an obvious dose-dependent trend. In this study, after establishing the OPF model in the observation group, after treatment with aspirin for 2, 4, 8, 12 weeks, BMP at each time point were significantly higher than that of control group (P<0.005), confirmed that aspirin can improve the BMP of OPF, has significant promoting effect on fracture healing in OPF rats. Other experiments further confirmed that[16], aspirin can improve the three-dimensional structure of bone trabecular in OP rats, increase the bone strength and density. The experiment results show that the 12 weeks of treatment, biomechanical strength of observation group was significantly higher than that of control group (P<0.05), and also confirmed that aspirin can increase the bone mineral density and mechanical strength in OPF rat; X-ray and histological observation in this study, at the 8th week, the endochondral ossification process of observation group was faster than that of observation group, with fuzzy fracture line in observation group and clear fracture line in observation group; at the 12th week, fracture line disappeared in observation group, fracture line of the control group was fuzzy at the same tim, also suggests that aspirin can accelerate fracture healing in OPF rats with distinct curative effect.

This study showed that Aspirin can accelerate the healing of new callus in OPF rats, increase bone density and biomechanics strength, and promote fracture healing of osteoporotic rats.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Wen TL, Sun TS, Wang L. Drug therapy of osteoporosis. *J People Army* 2010; (9): 42-44.

[2] Im F, Abrahamsson PA, Miller K. Preserving bone health in patients with hormone-sensitive prostate cancer: the role of bi-sphosphonates. *BJU Int* 2009; **104** (11): 1573-1579.

[3] Liu YT, Zhao XL, Wang XY, Bao L, Sa N, Wei CZ, et al. The research progress of osteoporosis treatment. *J Animal Husbandry Feed Sci* 2011; **32** (4): 127-128.

[4] Ceng BF, Li XL, Zhang ZL. The challenges of osteoporosis and osteoporotic fracture. *J Shanghai Med Sci* 2011; (3): 9-11.

[5] Yang H, Zhao HE, Wu GC, Wei GJ, Yang Z. Research progress of osteoporosis diagnosis and treatment of risk threshold and fracture. *J People Army* 2012; (11): 107-109.

[6] Kishimot B. Bisphosphonates role in cancer therapies. *Oral Maxillofac Surg* 2009; **67**(5 Suppl): 19-26.

[7] Chen ZW, Wu ZX, Sang HX, Wang LS, Feng J, Wang J, et al. experimental study of aspirin in osteoporosis treatment. *Chin J Med* 2011; **91**(13): 925-929.

[8] He T, Yang DC, Liu ZH. Diagnostic criteria of osteoporosis. *Chin J Osteoporosis* 2010; (2): 151-156.

[9] Wen TL, Sun TS, Wang L. Clinical manifestations and diagnosis of osteoporosis. *J People Army* 2010; (9): 40-41.

[10]Larsson S, Procter P. Optimising implant anchorage (augmentation) during fixation of osteoporotic fractures: Is there a role for bone-graft substitutes? *Injury* 2011; **42**(Suppl 2): 72-76.

[11]Li ZM. Clinical analysis of drug treatment for osteoporotic fracture. *J Clin Rational Drug Use* 2014; **7**(1): 51-52.

[12]Fonseca H, Moreira-Gon alves D, Coriolano HA. Bone quality: the determinants of bone strength and fragility. *Sports Med* 2013: 1-17.

[13]Ding H, Xu QZ, Deng G, Wang XY. The research progress of relationship between imaging and osteoporosis. *J Pract Geriatric Med* 2011; **25** (4): 280-283.

[14]Liao E. Status and progress of osteoporotic prevention and treatment. *Drug Evaluation* 2013; **10**(5): 15-21.

[15]Fitzpatrick LA, Dabrowski CE, Cicconetti G, Gordon DN, Papapoulos S, Bone HG 3rd, et al. The effects of ronacaleret, a calcium-sensing receptor antagonist, on bone mineral density and biochemical markers of bone turnover in postmenopausal women with low bone mineral density. *J Clin Endocrinol Metab* 2011; **96**(8): 2441-2449.

[16]Wang R, Sun LJ,Jiang HY. Effect of risedronate sodium on patients with postmenopausal osteoporosis bone mineral density and bone turnover. *Clin Focus* 2010; **25**(7): 607-608.

[17]John MR, Widler L, Gamse R, Buhl T, Seuwen K, Breitenstein W, et al. ATF936, a novel oral calcilytic, increases bone mineral density in rats and transiently releases parathyroid hormone in humans. *Bone* 2011; **49**(2): 233-241.

[18]Zhou J, He H, Yang L, Chen S, Guo H, Xia L, et al. Effects of pulsed electromagnetic fields on ipads mass and Wnt/beta - catenin signaling pathway in ovariectomized rats. *Arch Med Res* 2012; (4): 274-282.

[19]Tezval M, Biblis M, Sehmisch S, Schmelz U, Kolios L, Rack T, et al. The Animal models for ipads and be diseases. The ovariectomized and orchidectomized animals. *J Clin Calcium* 2011; **21**(2): 164-170.

[20]Horwitz MJ, Tedesco MB, Garcia-Ocana. A, Gundberg C, Garcia-Ocana A, Stewart AF. The Parathyroid hormone-related protein for the treatment of postmenopausal osteoporosis: defining the maximal tolerable dosed. *J Clin Endocrinol Metab* 2010; **95** (3): 1279-1295.

[21]Guo J, Liu M, Yang D, Bouxsein ML, Thomas CC, Schipani E, et al. The Phospholipase C signaling via the parathyroid hormone (PTH)/PTH - related peptide receptor is essential for normal ipads responses to PTH. *J Endocrinol* 2010; **151** (8): 3502-3151.

[22]Drake MT, Srinivasan B, Modder UI, Ng AC, Undale AH, Roforth MM, et al. Effects of intermittent parathyroid hormone treatment on osteoprogenitor cells in postmenopausal women. *J Ipads* 2011; **49**(3): 349-355.

[23]Cosman F, Lane NE, Bolognese MA, Zanchetta JR, Garcia-Hernandez PA, Sees K, et al. Effect of transdermal teriparatide administration on bone mineral density in postmenopausal women. *J Clin Endocrinol Metab* 2010; **95**(1): 151-158.

[24]Shoyele SA, Sivadas N, Cryan SA. The effects of excipients and particle engineering on the biophysical stability and aerosol performance of parathyroid hormone (1-34) prepared as a dry powder for inhalation. *AAPS Pharm Sci Tech* 2011; **12**(1): 304-311.

[25]Yang H, Wu GC, Wei GJ, Nong XL, Lu QT, Wei CM, et al. The plug phosphonic acid sodium with pulsed electromagnetic field therapy in treatment for castration rats with osteoporosis. *Chin J Osteoporosis* 2014; **20** (1): 67-72.