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YIXUE YINGYU

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郭云良　纪晓军　李益群　赵均发　马术魁　主编

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PREFACE

Since the founding of new China in 1949, the government has been paying high attention to traditional Chinese medicine (TCM) and pushing it developed to combine with Western medicine closely, and found a new discipline: Integrative Chinese-Western Medicine (Integrative medicine).

Integrative medicine is important for both Chinese and Western medical students. This textbook aims to make graduates to learn modern medical knowledge in English language, exchange and spread TCM internationally. So that they will combined Chinese and Western medicine to realize an integrative medicine in scientific meaning.

This textbook consists of 18 chapters and each consists of 4 parts. The first part is mainly Western medicine to culture English-Chinese and Chinese-English translating abilities for graduates from undergraduates of TCM, while the second part mainly Chinese medicine to educate those abilities for undergraduate from modern medicine. The third part is an original article of integrative medicine to exercise reading and writing abilities, while the forth part is Chinese-English vocabulary of TCM to help applying abilities.

This textbook involves Chinese medical theories of yin and yang, five elements, syndrome diffrentiation for treatment, meridians and collaterals, acupuncture and moxibustion, pushing and grasping, formula of traditional Chinese medicine and prescriptions, etc. And modern medicine includes basic knowledge of human system, mechanism and diagnosis and treatment for major diseases, etc. It could be satisfied for the graduates of integrative medicine, and might provide a reference book for associated discipline's researchers.

We thank Dr. Long Shaohua, Li Xiaodan, Zhao Li, Bao Hong, Hao Zhimin, He Xinze, Jiang Wen, Gao Yuanyuan, Sun Binbin and Zhang Kaitai for editing this textbook.

We also thank Qingdao University Medical College and Affiliated Hospital for supporting publish this textbook.

There will be weaknesses under correction, we thank your for your comments.

EDITORS

2013.08

前　言

20世纪中叶新中国成立以来，我国政府对中医学给予了高度的重视，极大地推动了中医学的发展，并将中医学和西医学有机结合，形成了一门新的科学体系——中西结合医学。

西医学生不懂中医是很大的不足，中医学生不懂西医也将寸步难行。本教程目的旨在使中西医结合专业研究生通过英语语言工具获取西医学的知识，以专业英语为桥梁，进行国际交流，传播中医。从而使中医和西医专业知识揉为一体，相互借鉴、取长补短，实现真正意义上的中西结合医学。

本教材分18章，每章包括四部分。第一部分主要为西医内容，以培养和锻炼本科为中医专业的研究生的英语阅读理解（英译汉）能力和写作（汉译英）能力。第二部分主要为中医内容，着重于培养和锻炼本科为西医专业的研究生的阅读理解能力和英语写作能力。第三部分系一篇中西医结合研究论文，培养和锻炼学生的专业阅读和写作能力。第四部分为中西医结合专业名词的中英文对照，以便使研究生在翻译和写作时能正确应用。

本书中医内容包括阴阳五行、八纲辨证、经络腧穴、针灸推拿、中药方剂等。西医内容包括各器官系统的基础知识，主要疾病的病因、发病机制和诊疗方法等。内容由浅入深、循序渐进，从医学科普知识，逐渐深入到专业论文的翻译和写作，最终使研究生具备能顺利阅读和翻译（双向翻译）本专业英文文献的能力，可满足中西医结合相关专业研究生的需要，也可供相关专业研究人员参考使用。

除编写委员会外，参加编写的人员还有龙少华、李晓丹、赵丽、包红、郝志民、贺新泽、姜文、高媛媛、孙彬彬、张开泰等。

在编写过程中，青岛大学医学院及附属医院给予了支持，表示感谢。

由于编者水平有限，书中难免存在不足之处，恳请读者指正。

编　者

2013.08

CONTENTS

CHAPTER ONE

Section 1 Fracture and Osteoporosis

A fracture can be more than just a broken bone. It may be a warning sign of osteoporosis or "porous bone", a medical condition that weakens bone by making it more porous and less dense. Bone density is one of the factors that determine bone strength, so individuals with low bone density have a higher risk for fracture and refracture.

Osteoporosis is a contributing factor in as many as 1.5 million fractures each year, including about 300,000 hip fractures, 700,000 vertebral (spine) fractures, 250,000 wrist fractures and 300,000 fractures at other sites.

The risk of a serious fracture can double after a first fracture in certain high-risk groups. Additionally, many patients, particularly those who suffer hip fractures, are at high risk for premature death or loss of independence after the fracture. In facts, one out of four people who have an osteoporotic hip fracture will need long-term nursing home care; half of those who experience osteoporotic hip fracture are unable to walk without assistance; those who experience the trauma of osteoporotic hip fracture have a 24% increased risk of dying within one year following the fracture. So it is essential for indentifying osteoporosis early and initiating treatment.

Recent data indicates that osteoporosis should not just be concern for aging white women, but occurs in all racial groups. (1)Hispanic women may be among those at highest risk of osteoporosis with 13%-16%. As many as 49% of Mexican-American women 50 years of age or older have low bone density. (2)Although the rate of hip fracture is lower in Asian-American women, the rate of vertebral fractures is about equal between Asian-American and Caucasian women. (3) About 10% of African women over 50 years have osteoporosis. An additional 30% have low bone density. About 80%-95% of all fractures experienced by African-American women over age 64 are related to osteoporosis.

Men should also be concerned about osteoporosis. Approximately 1/8 men will have an osteoporotic fracture. Men with a history of hypogonadism, thyroid dysfunction, long-term steroid therapy, high alcohol consumption or low physical activity are especially at risk. One-third of all hip fractures experienced by men are related to osteoporosis, and 1/3 of these men will die within the first year after the fracture.

A fracture in adulthood does not always mean an individual has osteoporosis. However, every adult who suffers a fracture should discuss the need for bone density testing with a physician. If one's bone density is low, he may need additional medical tests. Medical conditions other than osteoporosis can cause low bone density.

Although there is no specific cure for osteoporosis, diet and lifestyle changes can reduce the risk of refracture. People should also discuss medical therapy with their physician. Even individuals without osteoporosis should follow these 4 simple guidelines: (1) Make sure get enough calcium and vitamin D in diet. The National Academy of Sciences recommends 400-800 units of vitamin D and 1,000-1,500 mg of calcium per day. (2) Participate in activities that will strengthen bone and muscle. Regular exercise is one of the best things to prevent one's osteoporosis. Weight-bearing exercises like walking, jogging and tennis and low-impact exercise classes are best for building and maintaining strong bones. (3) Because falls are the most common cause of fractures, some balance activities could reduce the risks. The benefits of *tai chi* which can decrease falls among older individuals by 47% in particular have been documented. (4) Once a broken bone occurred, a bone density test and other steps to reduce the risk of a second fracture should be done.

Section 2 Nomenclature in TCM

Correctly naming discipline can directly portray the object of study, clearly point out its essence, and presage its future development. Carefully naming an independent discipline may provide the breakthrough necessary to establish its independence. Given the importance of nomenclature, the terminology being applied to the various branches of Traditional Chinese Medicine (TCM) is disconcerting. Current usage of such terms as "Chinese medicine", "oriental medicine", "herb", "herbology", "herbal medicine" and "acupuncture" are improper. The present article aims to discuss these terms and definite their meaning more precisely.

1. Regarding "Chinese Medicine"

The traditional medicine of China is the classical medicine of China and is distinguishable from modern Chinese medicine. TCM is a medical science guided by traditional Chinese medical theories, and includes natural product medication, acupuncture, moxibustion, massage, plaster, steam bath, etc. as modalities in the treatment and prevention of disease.

TCM is called "zhongyixue" (phonetic transcription of Chinese character, the same below) in Chinese. The Chinese characters "zhongyixue" and "zhonghua yixue" are both translated literally into the English words "Chinese medicine". However, to refer to TCM as "Chinese medicine" has two shortcomings. First, "Chinese medicine" fails to convey the rich "tradition" associated with TCM; and second, this translation leads to confusion by not distinguishing between TCM and modern Chinese medicine. Examples of the confusion associated with the global term "Chinese medicine" include the following. Currently, some books and journals published in English include "Chinese medicine" in their titles; however, are concerned only with TCM. On the other hand, another journal entitled "Chinese Medical Journal" is concerned only with modern Chinese medicine. Although the above titles "Chinese medicine" suggests that they contain similar material, the material in fact is quite different. In addition, some institutions with the name "Chinese medicine" in fact are concerned only with TCM, not modern Chinese medicine. Finally, an organization named "Chinese Medical Association" is a society involved only with modern medicine in China. The subject material of "Chinese medicine" as used above differs so extensively that TCM and modern Chinese medicine must have a distinguished nomenclature.

Medicine in the broadest sense should include both modern medicine and traditional medicine. However, the term, "medicine" typically refers only to modern medicine. The word "traditional" is often used to distinguish traditional medicine from (modern) medicine. For these reasons, "zhongyixue" should be translated into English according to its precise definition, "traditional Chinese medicine". This translation conveys both the traditional and the Chinese aspects of the discipline, and distinguishes it from modern Chinese medicine.

2. Regarding "Oriental Medicine"

Another vague, ambiguous term is "oriental medicine". "Oriental medicine" in English publications is usually synonymous with traditional medicine of China, Japan, Korea, Vietnam, etc. Actually, all of these disciplines are various branches of TCM which according to relevant literature originated in China. Moreover, in Japanese, traditional medicine is called "Han Fang", which translates into TCM. The term "oriental medicine" probably originated to distinguish it from western "occidental medicine" which is usually associated with modern medicine since it originated in the West. Although the "oriental" or "occidental" qualifier may be convenient to emphasize the origin of particular aspects of medicine, such use is detrimental because of its ambiguity. The use of the "oriental" qualifier has at least two shortcomings: it fails to express its original meaning of traditional medicine in China, and it also fails to recognize the existence of modern medicine in the East. The use of the "occidental" qualifier bears the same kind of shortcomings. It does not convey the difference between occidental traditional medicine and modern medicine. If medicine were referred to as "occidental medicine" as currently is done with "oriental medicine", differentiation of modern and classical Western medicine such as Hippocratic medicine in 6-4 centuries B. C. and American Indian folk medicine would be compromised. Though the classical medicine in the west has gradually withered away, it existed in history and needs to be distinguished just as traditional Chinese medicine needs to be distinguished from modern Chinese medicine.

Difference in the nature of medicine in the west and the east has been dissolving for a long time. Currently only differences in tradition and degree of development distinguish the two. With time, the disparity in development between western and eastern medicine will become smaller and smaller, leaving only tradition as a distinction. Since the essence of "oriental medicine" is traditional Chinese medicine, its true colors of TCM should be restored.

To name disciplines of medicine by using the "oriental" or the "occidental" qualifier can not directly portray the object of study, clearly point out its essence, and presage its future prospects of growth. Neither differentiates traditional medicine nor modern medicine in either the West or the East. If the word "oriental" is necessary to precede traditional medicine in the east, it can only be named traditional oriental medicine to differentiate (modern) medicine in the East. This reason is the same as that the naming of TCM is differentiated with (modern) medicine in China.

3. Regarding "Herb", "Herbology" or "Herbal Medicine"

Traditional Chinese Materia Medica (TCMM) is a branch of TCM, which studies the theory and application of medication based on theories of TCM. TCMM differs extensively from herbal medicine. The relationship between the two is somewhat akin to the difference between folk medicine and natural product pharmacy in the US. It is improper to use TCMM, synonymously with the terms herbology or herbal medicine. In China, TCMM is an officially recognized branch of TCM with standardized medication procedures documented in the state pharmacopoeia or equivalent books. Herbal medicine, however, is folk medicine, and is not officially recognized in the state pharmacopoeia. The two procedures are much different with TCMM having a systematic theory as guidance for its practice. In TCMM, medicines are derived mainly from plants, but animals and minerals provide additional sources. The materials are prepared and refined using well established procedures. The English phrase herbal medicine generally refers to folk medicine, and in most cases the crude herb or crude extract is used. Animal products and minerals are generally not included in definitions of herbal medicine.

For the above reasons, to translate Chinese word "zhongyao" —— TCMM into herb, herbology or herbal medicine is improper on either the basis of the definition of TCMM or the English meaning of the world herb.

4. Regarding "Acupuncture"

The science of acupuncture and moxibustion is a branch of TCM which prevents and treats diseases by puncturing specific points on the body with needles, or the burning or warming of the points by applying heat via ignited moxa wool or roll. These procedures are important external therapies of TCM. The term acupuncture is derived from the Latin words *acus* which means surgical needle and puncture. Moxibustion can be defined as the burning, warming, fumigating, or placing hot compressions on certain points for the treatment or prevention of diseases. The two therapies are commonly applied in combination, and are also typically used as compound word acupuncture-moxibustion (acumoxibustion —— Chinese sound "zhenjiu", which literally means "needling-moxibustion").

In the literal sense, acupuncture refers to a method of needle (i.e. Chinese words "zhenci") or a therapy of needling (i.e. Chinese words "zhenci liaofa"); however, science of acupuncture and moxibustion is also a discipline (i.e. Chinese words "zhenjiuxue"), a branch of TCM. When using the term "acupuncture" we should make clear whether we are referring to its meaning as a method, a therapy or a scientific discipline. Use of the term "acupuncture" without distinguishing among these three meanings leads to a confusion that could be eliminated by refining the use of the term. In this light, it is proposing that when using "acupuncture" to refer to the discipline, we should use the term "acupunctology" (i.e., "acupuncture" plus "-ology"). This term should become the standard through international scientific meetings. It is assumed that acupunctology in the broad sense could cover moxibustion and such similar therapies in the same field.

In summary, the present paper suggests that the current use of "Chinese medicine", "oriental medicine", "herb", "herbology" or "herbal medicine" and "acupuncture" as terms representing disciplines of TCM and its branches is either improper or indistinct. Without more attention to concisely defining these terms, many individuals consider that TCMM is a folk herbology, and acupuncture as only a therapeutic method. Under such conditions, TCM is demoted to folk medicine; the science of TCMM to herbology or herbal medicine; the doctor of TCM to herbalist; the science, acupunctology, to the therapy acupuncture; and the acupunctologist to the acupuncturist. The present paper is a call to recognize TCMM and acupuncture as scientific branches of TCM with appropriate translations to reflect their scientific nature and separate them from folk therapies.

Section 3 Original Article

Zhuangjin xugu decoction enhances fracture healing in rats by augmenting the expression of BMP-7 and NPY

[Abstract] This study aimed to investigate the effects of Zhuangjin xugu decoction (ZJXG decoction) on healing of femoral fracture in rats. Femur fractures were generated in fifty male adult *Wistar* rats by cutting femur transversely at middle point. ZJXG Decoction was administered orally after surgery for 7-14d. The healing process was analyzed by X-ray and hematoxtlin-eosin (HE) staining in rats of sham group, control group and treatment group. The expression of bone morphogenetic protein-7 (BMP-7) and neuropeptide Y (NPY) in fibroblasts and osteoblasts in callus was evaluated by immunohistochemical assay. The serum levels of BMP-7 and NPY were detected by enzyme linked immunobsorbent assay (ELISA). X-ray imaging analysis indicated that the fibrous callus tissue at the femoral fracture-end increased and the fracture line became fuzzy at 7-14 d following treatment with ZJXG Decoction, compared to control group. HE staining showed that the fibrous-granular tissue at the fracture-end changed gradually to fibrous, cartilaginous and osseous callus tissues. Immunostaining and ELISA results showed that BMP-7 and NPY in the fibroblasts and osteoblasts of callus and their serum levels increased significantly 7-14 d following treatment with ZJXG Decoction, compared to control group. It is concluded that ZJXG Decoction could enhance the fracture healing by up-regulating the expression of BMP-7 and NPY in fibroblasts and osteoblasts of callus in rats.

[Key words] ZJXG Decoction; fracture; callus; X-ray; pathology; BMPs; NPY; rats

1. Introduction

The fracture healing is an extremely complicated process of skeletal reconstruction. Many growth factors could promote osteoblast differentiation, proliferation, development and accelerate new bone formation in the process of fracture healing and remodeling (Pogoda, et al. 2005). Bone morphogenetic proteins (BMPs) and neuropeptide Y (NPY) play important roles in bone fracture repair process (Oreffo RO. 2004). BMP-7 can induce cartilage and bone formation (Chen, et al. 2002) and has been used to treat fracture models in rodents (Hak et al., 2006; Lu et al., 2010; den Boer et al., 2002). Some experimental and clinical reports illustrated the effect of BMP-7 on fracture healing (Blattert, et al., 2002; White et al., 2007). NPY affects the activities of osteoblasts by inhibiting the synthesis of circle adenosine monophosphate (cAMP) during the fracture repair process (Linblad, et al., 1994). As a skeletal maintenance regulation-related factor, NPY improves skeletal synthetic metabolism, controls bone remodeling, regulates bone balance, prevents bone loss, and maintains bone stability (Lee N J, Herzog H. 2009; Teixeira, et al. 2009). NPY participates in fracture remodeling not only from the central nervous system, but also from the tissue surrounding fracture (Liu, et al. 2009).

Current fracture care includes internal and external fixation with early mobilization to restore function earlier and more completely. But fracture fixation could cause serious trauma and mostly need the secondary operation. In addition, it increases the risk of infection and the rates of delayed union, and nonunion (Zhao, et al. 2011). Traditional Chinese medicine formula "Zhuang Jin Xu Gu Decoction (ZJXG Decoction)" has been clinically used for promoting fracture healing for many years (Li K, et al. 2009). The exact therapeutic mechanism by which ZJXG Decoction enhances healing in rodent model, however, still remains unclear. Here we aimed to elucidate if the effects of ZJXG Decoction in fracture repair was related to the expression of BMP-7 and NPY.

2. Materials and Methods

2.1. Animal Model and Grouping

Fifty male adult *Wistar* rats (Experiment Animal Center of Qingdao Drug Inspection Institute, SCXK (LU) 20090010) weighting 190-210g were used in this study. All experimental procedures were approved by the Ethics Committee of Qingdao University Medical College (No. QUMC 2011-09). The rats were anesthetized with injecting intraperitoneally 100g/L chloral hydrate (300mg/kg) and then restrained in a supine position for operation. The animal's hind limb was shaved and then a medial prepatellar incision was created. The femoral fracture model was established by cutting the femur transversely at the middle section (about 1.0 cm below the great trochanter) (Wang, et al., 2005). After the manual reduction the fractured femur was fixed with intramedullary Kirschner wires (diameter 1.0 mm, Shanghai Medical Apparatus Co. Ltd.). The sham group was subjected to the same procedure except without cutting femur. Animals were allowed to drink and eat freely after surgery. The survival rate is 100%.

The rats were divided randomly into five groups of 10 rats in each group. The low, medium and high dose group rats were treated with 1.25, 2.50 and 5.00 g/kg respectively while the vehicle was given at the same volume to sham and control group rats. At the time points of 7d or 14d, rats were subjected to X-ray image taking after chloral hydrate anesthesia and then euthanized for blood and tissue collection.

2.2. Preparation of *Zhuangjin Xugu Decoction*

ZJXG Decoction was derived from the ZJXG Pellet recorded in "Shangke Dacheng" written by Zhao Lian of the Qing Dynasty in China. It is composed of 12 constituents (Chinese herbal medicines) listed in table 1.

The ZJXG Decoction was decocted according to the Standard of Decocting Herbal Medicine promulgated by Chinese Administration Department of Traditional Chinese Medicine. The mixture of all herbal plants were immersed in distilled water for 20-30 min at 20-25℃ with relative humidity ≤85%, and then cooked to the boil, kept on simmer for 10-15 min to concentrate the extracts, protecting and maintaining all essential ingredients. The same procedure was repeated for 2 times. The two extractions yielded an amount of 224 ml liquid medicinal decoction containing 112g of dry weight (concentration of 0.50 g/ml) which was packed with sterilized plastic bags and stored at −20℃ until use.

Tab.1 Chinese herbal medicines of ZJXG Decoction

Sources	Chinese name	English Name	*Lating Name*	Dose
Gansu	Danggui	Chinese Angelica	*Radix Angelicae Sinensis*	12g
Sichuan	Chuanxiong	Rhizoma Chuanxiong	*Rhizoma Chuanxiong t*	12g
Henan	Shudi	Radix Rehmanniae Preparata	*Radix Rehmanniae Preparata*	10g
Inn Mongolia	Huangqi	Milkvetch Root	*Radix Astragali*	12g
Sichuan	Duzhong	Eucommia Bark	*Eucommia ulmoides Oliv*	12g
Sichuan	Chuanxuduan	Himalayan Teasel Root	*Radix Dipsaci Asperoidis*	12g
Guangxi	Gusuibu	Fortune's Drynaria Rhizome	*Rhizoma Drynariae*	12g
Yunnan	Sanqi	Sanchi	*Radix Notoginseng*	10g
Inn Mongolia	Baishao	White Paeony Root	*Radix Paeoniae Alba*	10g
Xinjiang	Honghua	Safflower	*Flos Carthami*	10g
Total				112g

2.3. Radiological Evaluation and Gross Observation

An initial X-ray examination was performed in all animals after the fracture. At 7 and 14 d following surgery, all the rats were anesthetized for X-ray evaluation (GE Revolution RE/d, USA). The anesthetized rats were then sacrificed and the femurs were taken out, washed normal saline for general observation.

2.4. Histological Analysis

For morphological analysis, the femur were cut and incubated in 40g/L formaldehyde solution for 4 h and rinsed in distilled water for 4h, and then decalcificated for 10 days in 20% ethylenediamine tetraacetic acid (EDTA). The samples were then dehydrated using graded ethanol, immersed in dimethylbenzene for 2h, embedded by paraffin. The 7μm thickness slices were made by mirotome (Leica RM 2015, Shanghai Leica Instruments, China) and attached to poly-L-lysine processed slides. Paraffin sections were deparaffinaged in dimethylbenzene, hydrated in gradient ethanol and rinsed with distilled water. The sections were stained with hematoxylin-eosin and BMP-7 and NPY.

For immunostaining, antigen retrieval was made using a microwave oven. The sections were incubated with Rabbit anti-rat BMP-7(concentration) and NPY (concentration) polyclonal antibodies at 4℃overnight. Negative control used PBS instead of primary antibodies. Immunohistochemical procedures were performed strictly according to the SABC kit manual. Four serial sections from each experimental rat were observed under a light microscope (manufacture). LEICA Qwin micrograph analytical system was used to analyze the expression of immuno signals, illustrated by absorbance values (*A*).

2.5. Enzyme Linked Immunosorbent Assay (ELISA)

About 4 ml blood was aseptically collected from abdominal aorta of each rat and centrifugalized for 10 minutes at 4000 r/min at 4 ℃ to separate the serum which was then kept at −20 ℃ until required for analysis. BMP-7 and NPY were measured using commercially available ELISA kits (Blue Gene Co. Ltd). The procedure was performed following manufacturer's instruction. The ODs were calculated with Bio-Rad 550 microplate reader (USA) set to 450nm to reflect the level of BMP-7 and NPY.

2.6. Statistical Analysis

The data was expressed by mean ± standard deviation ($\bar{x} \pm s$) and analyzed with SPSS 11.5 statistical software. Analysis of variance was used to compare whether there are obvious differences among groups. $P<0.05$ was considered significantly.

3. Results

3.1. X-ray Examination

X-rays revealed that the fracture-end of femur of the control group began forming fibrous callus at 7 day after surgery with the fracture line still clear; at 14 days, the fracture line became unclear. In the treated groups, the fibrous callus was more than that in the control group and the fracture line became fuzzy at 7 days and tended to disappear at 14 days following treatment (Figure 1). There was no statistical significance between groups of low dose, medium dose and high dose group.

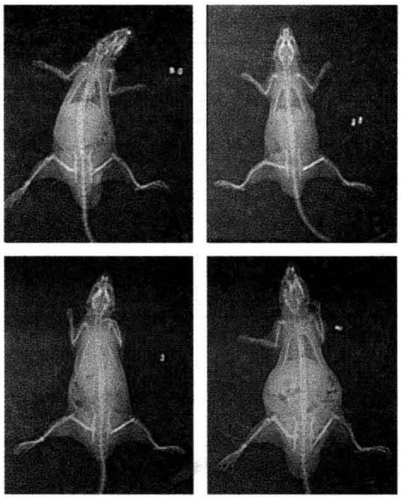

Fig.1 The X-ray films of femur fracture healing on day 7 in control group (A) and low-dose treated group (B), day 14 in control group (C) and low-dose treated group (D)

3.2. Gross Observation of Fracture Fragments

On day 7 in control group, granulation tissue in the fracture breaking-end was observed and fibrous callus at 14 days following surgery. In the treated groups, fibrous callus formed at 7 days and formation of cartilaginous and osseous callus was present on day 14 (Figure 2). There was no statistical difference among treatment groups.

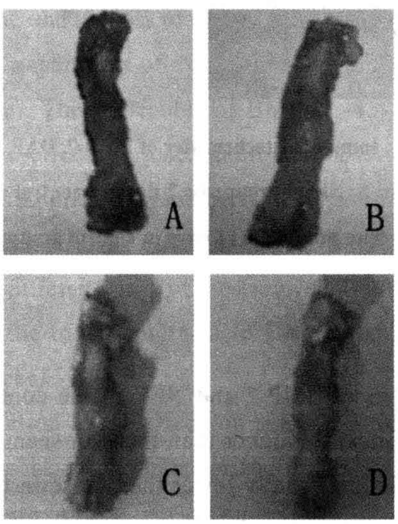

Fig.2 Gross observation of tissue samples on day 7 in control group (A) and low-dose treated group (B), day 14 in control group (C) and low-dose treated group (D).

3.3. HE Staining

On day 7, in control group, the inflammatory cell infiltration, formation of granulation tissues occurred between fracture fragments, and the proliferation of fibroblast and osteoblasts under periosteum was localized in the fracture gap. On day 14 of control rats, the number of fibroblasts and osteoblasts increased and fibrous callus had formed with a small cartilaginous callus. In the treated groups, the inflammatory cells decreased and the fibroblasts and osteoblasts increased in the fractured bone end 7 days after treatment compared to control , while on day 14 a lot of fibrous, cartilaginous and osseous callus tissues had developed and newly formed bone trabeculae appeared (Figure 3).

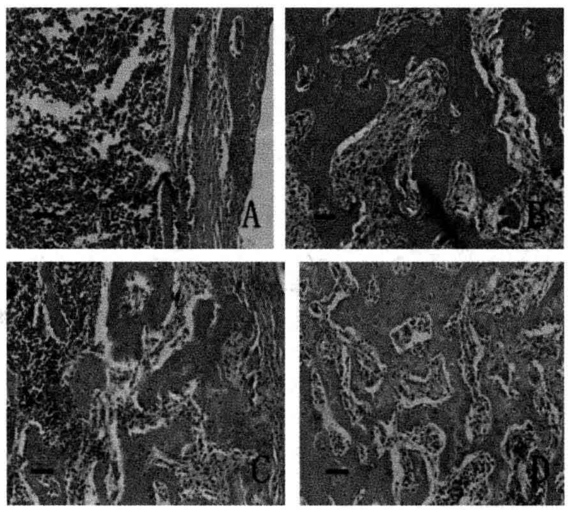

Fig.3 Hematoxylin and eosin staining on tissues collected on day 7 in control group (A) and treatment group (B), on day 14 in control group (C) and treatment group (D). Scale bar =50μm.

3.4. Immunohistochemistry

Minimal expression of BMP-7 and NPY was detected in the sham group (F=14.12, q=2.39-7.69, P >0.05). BMP-7 and NPY positive cells were observed in callus tissues in control group on day 7 and the absorbance values on day 14 was greater compared to 7d control group (F=14.12, q=2.39-7.69, P<0.05). In paired comparisons of groups, the grade of values of absorbance (A) of BMP-7 and NPY was significantly higher in the treatment groups compared to control group (F=14.12, *q*=2.39-7.69, *P*<0.05). It was not significantly different among the high-dose, medium-dose and low-dose treated groups (*F*=14.12, *q*=2.39-7.69, *P*>0.05) (Table 2 and Figure 4, Figure5).

Tab.2 The expression values of absorbance (*A*) of BMP-7 and NPY(X±S, n=5)

Groups	Dose	BMP-7 (*A*)		NPY (*A*)	
		7 d	14 d	7 d	14 d
Sham group	NS	0.24 ± 0.07	0.25 ± 0.06	0.27 ± 0.05	0.25 ± 0.07
Control group	NS	0.28 ± 0.06^{a}	0.43 ± 0.08$^{a c}$	0.38 ± 0.06^{a}	0.46 ± 0.11$^{a c}$
Low-dose group	1.25g/kg	0.65 ± 0.12^{b}	0.71 ± 0.14$^{b c}$	0.56 ± 0.10^{b}	0.70 ± 0.10$^{b c}$
Medium-dose group	2.50g/kg	0.62 ± 0.12^{b}	0.81 ± 0.12$^{b c}$	0.57 ± 0.12^{b}	0.71 ± 0.13$^{b c}$
High-dose group	5.00g/kg	0.63 ± 0.13^{b}	0.83 ± 0.15$^{b c}$	0.57 ± 0.16^{b}	0.68 ± 0.13$^{b c}$

^{a} *P*<0.05 *vs* sham group, ^{b} *P*<0.05 *vs* control group, ^{c} *P*<0.05 *vs* treated 7 d

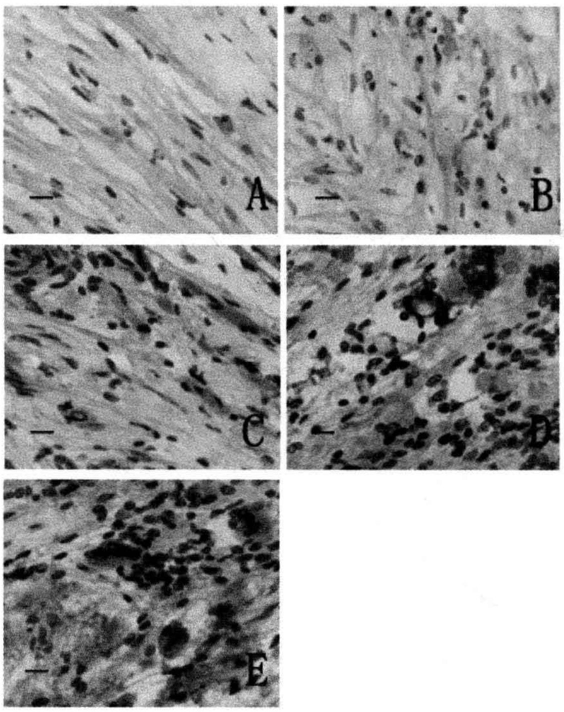

Fig.4 The expression of BMP-7, DAB × 200, Scale Bar = 25μm. A: Sham group; B: 7 days in control group; C: 7 days in low-dose group; D: 14 days in control group; E: 14 days in low-dose group

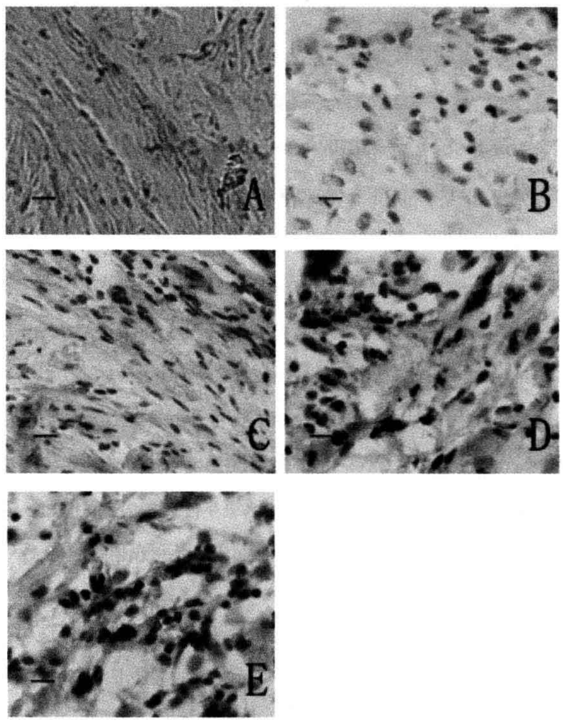

Fig.5 Immunohistochemistry of BMP-7, DAB × 200, Scale Bar = 25μm. A: Sham group; B: 7 days in control group; C: 7 days in low-dose group; D: 14 days in control group; E: 14 days in low-dose group

3.5. The serum Levels of BMP-7 and NPY

There was no significant difference of serum levels of BMP-7 and NPY between day 7 and day 14 in sham operation group (*P*>0.05). It is significantly different between day 7 and day 14 within other each group, with higher levels on day 14 (*P*<0.05). At same time points, the serum levels of BMP-7 and NPY in the control group were significantly higher than those in sham operation group and significantly lower than those in the treated groups (*P*<0.05). No significant difference among the high-dose, medium-dose and low-dose groups was observed (*P*>0.05) (Table 3).