Effect of kidney-reinforcing and marrow-beneficial Chinese medicine on bone metabolism-related factors following spinal cord injury in rats

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Abstract. The present study aimed to investigate the effect of traditional Chinese kidney reinforcing and marrow-beneficial medicine (KRMB) on the prevention and treatment of abnormal bone metabolism and osteoporosis (OP) resulting from spinal cord injury (SCI). Rat models of OP following SCI were surgically established. The rats were randomly divided into five groups: Normal; sham operation + KRMB; normal + KRMB; SCI + KRMB; and SCI model group. Bone mineral density (BMD), and the expression of bone gamma-carboxyglutamic-acid containing protein (BGP), hepcidin mRNA and bone sialoprotein (BSP) were recorded at 1, 2, 4, 6, 8 and 10 weeks after the operation. BMD expression in the SCI model group was significantly lower compared with the normal, sham + KRMB and normal + KRMB groups at 4, 6, 8 and 10 weeks (P<0.01), and was significantly lower than that in the SCI + KRMB group at 6 (P<0.05), 8 and 10 weeks (P<0.01). The level of serum BGP in the SCI model group was significantly higher compared with the normal, sham + KRMB and normal + KRMB groups at each time point (P<0.01), and lower than the SCI + KRMB group (P<0.01). The SCI + KRMB group was significantly higher than the normal, sham operation + KRMB and normal + KRMB groups (P<0.01). Hepcidin mRNA expression in the rat livers in the normal, sham + KRMB and normal + KRMB group was significantly higher than that in the SCI + KRMB group and SCI model group at each time point (P<0.01). Hepcidin mRNA expression in the SCI + KRMB group was significantly higher than that in the SCI model group at 1 week (P<0.01), and significantly higher than the SCI model group at 2, 4, 6 ,8 and 10 weeks (P<0.01). BSP expression in the SCI model group was significantly higher than that in the normal, sham + KRMB and normal + KRMB groups at each time point (P<0.01). BSP expression in SCI model group was higher than that in the SCI + KRMB group at 1 (P<0.05), 2, 4, 6, 8 and 10 weeks (P<0.01). In conclusion, KRMB traditional Chinese medicine may have a curative effect on secondary OP resulting from SCI.

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Key words: kidney-reinforcing and marrow-beneficial traditional Chinese medicine, spinal cord injury, bone metabolism, bone metabolism related factors

Introduction

Spinal cord injury (SCI) is a catastrophic injury that has a high disability rate and effects feeling, movement and autonomic functions, and has a number of serious secondary life-threatening complications (1). As society develops, the incidence of SCI increases (2). Osteoporosis (OP) is a primary complication of SCI, affecting primarily patients below the level of injury, and increases the probability of fracturing lower limbs (3). The pathological mechanism that causes secondary OP is currently uncertain, and there is, therefore, a lack of systematic and efficient treatment.

According to traditional Chinese medicine theories, the main pathogenesis of OP resulting from SCI is marrow deficiency and kidney asthenia (4,5). Therefore, kidney reinforcing and marrow-beneficial (KRMB) traditional Chinese medicines may be prescribed (6). A number of studies have demonstrated that KRMB can significantly increase bone mineral density (BMD) in rats without ovaries, improve bone tissue, and promote the growth and differentiation of osteoblast (OB) cells (7). In addition, it has been reported that KRMB intervention in rats (weight, 28.125 g/kg; gavage 1 h later; 25% concentration) promotes bone marrow stromal cell proliferation and osteogenic differentiation (8).

The present study aims to investigate the effect of KRMB on kidney and bone marrow metabolism-related factor expression following SCI, and to study the pathomechanism of SCI and OP. This may lay the foundation for the prevention and treatment of OP resulting from SCI using traditional Chinese medicine.

Materials and methods

Animals. A total of 240 pathogen-free Sprague-Dawley rats (weight, 200±20 g; 120 male and 120 female; age, 3 months)

were obtained from the Experimental Animal Center of Chinese Medical Sciences University (Shenyang, China). Animals were maintained in grouped-housing in a temperature (20-25˚C) and humidity (40-55%) controlled environment, with a 12 light/dark cycle and *ad libitum* access to food and water.

Preparation of reagents. KRMB was prepared as a suspension containing 10 g lyophilized powder of fresh antler (Animal Husbandry of Shunda, Jilin, China), 5 g oyster powder and 15 g *Epimedium brevicornum* decoction (both purchased from Jinzhou pharmacy market, Jinzhou, China) and refrigerated at 4˚C.

Drug administration. Rats were allocated at random into the following groups (n=4 per group): Normal; sham + KRMB; normal + KRMB; SCI + KRMB; and SCI model groups. The KRMB dose was 28.125 g/kg body weight (suspension volume, 1 ml), and the normal group was administered an equivalent volume of saline for 10 weeks, once a day, by gavage. Following the experiments, rats were sacrificed by an anesthetic overdose (10% chloral hydrate; 300 mg/kg; China Shanghai National Medicine Group Corporation, Shanghai, China).

Surgical procedure. Rats were fasted for 24 h, with free access to water, prior to the operation. Rats were anesthetized with an intraperitoneal injection of 10% chloral hydrate (300 mg/kg) and laid in the prone position. The thoracic T9-11 vertebra was marked as the center, and an aseptic operation along the spinous process was performed. A longitudinal incision (~4 cm) was made, blunt separation stripped the fascia, fat and paravertebral muscle, bite T7-9 spinous process and a laminectomy was performed on the T8 vertebrae in order to fully expose the back and sides of the dural sac. The endorachis and spinal cord were entirely transected using a 10 scalpel (Jinzhou Medical Instruments Factory, Changchun, China), and rat hind limbs convulsed several times prior to flaccid paralysis. Next, a 2-mm incision was made through spinal cord tissues below the T10 spinal segment, and a gelfoam sponge (Jinzhou Medical Instruments Factory) was placed at the broken ends of spinal cord. The endorachis was opened by incision and covered with a fasciai patch, and sutured layer by layer. The sham operation cut off the spinous process and lamina to expose the spinal cord, but there was no resection to the spinal cord (9-11). At 1, 2, 4, 6, 8 and 10 weeks after the surgery, 8 rats were randomly selected from each group and specimens were collected.

Once blood was collected from the rats, eliminated attachment of the muscle fascia, retained periosteum, taken the left hind limb, flushed by the physiological saline and preserved at -80˚C. BMD expression in the rat distal femur was detected using Lunar Prodigy dual-energy X-ray absorptiometry (GE Healthcare Life Sciences, Chalfont, UK). Post-injury motor behavior is assessed using the Basso, Beattie and Bresnahan (BBB) locomotor scale method (12). Rats are placed on an operating table to observe the hip joint, knee joint, ankle joint, the movement and coordination of walking, the torso and the tail. Rats were analyzed for 4 min between 8 and 9 p.m. following micturition. The average score of the rats' hind legs was then recorded using a single blind method.

Detection of bone gamma-carboxyglutamic-acid containing protein (BGP) expression. Rats were anesthetized with an intraperitoneal injection of 10% chloral hydrate (300 mg/kg), blood samples were extracted from the abdominal aortic separation and blood serum was separated by centrifugation for 10 min at 3,000 x g, and preserved at -80˚C. BGP expression was detected using an Osteocalcin (BGP) enzyme linked immunosorbent assay (ABE20719; R&D Systems China Co., Ltd., Shanghai, China) according to the manufacturer's instructions.

Detection of hepcidin mRNA expression. Hepcidin mRNA expression in the liver was determined using an RNA polymerase chain reaction (PCR) kit (AMV) (version 3.0; Takara Biotechnology Co., Inc., Dalian, China) and a 600 bp DNA ladder marker (Beijing TransGen Biotech Co., Ltd., Beijing, China). Primer Premier version 5.0 software (Premier Biosoft International, Palo Alto, CA, USA) was used to design PCR primer sequences for β-actin and hepcidin, based on the rat β-actin and hepcidin gene sequences registered in GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Subsequently, ~100 mg fresh rat liver tissue was homogenized in liquid nitrogen. Total RNA (1 μl) extraction was performed using TRIzol reagent (Takara Biotechnology Co., Inc.) according to the manufacturer's instructions. A reverse transcription-PCR (RT-PCR) kit (Takara Biotechnology Co., Inc.) was used to synthesize the first strand of cDNA. The reaction conditions were as follows: 42˚C for 30 min, 99˚C for 5 min, 5˚C for 5 min followed by preservation at 4˚C. The reaction mixture (total volume, 10 μl) contained 2 μl $MgCl_2$, 1 μl 10X RT Buffer, 3.75 μl RNase Free dH_2O, 1 μl dNTP mixture (10 mM), 0.25 μl RNase inhibitor, 0.5 μl AMV Reverse Transcriptase, 0.5 μl Oligo dT and 1 μl RNA. The hepcidin PCR protocol began with initial denaturation for 5 min at 94˚C, followed by amplification for 30 sec at 94˚C for 30 cycles, and 10 min at 72˚C. The β-actin PCR protocol began with initial denaturation for 40 sec at 72˚C, followed by an amplification program for 30 s at 55˚C. PCR products were electrophoresed using a 3-μl DNA ladder marker (DL600) with molecular weight standards (100 bp) as the reference. Electrophoresis was performed at 90 V for 1 h. The primer sequences were as follows: β-Actin forward, 5'-GGAGATTACTGCCCTGGCTCCTA-3' and reverse, 5'-GACTCATCGTACTCCTGCTTGCTG-3'; and hepcidin forward, 5'-GAAGGCAAGATGGCACTAAGCA-3' and reverse, 5'-TCTCGTCTGTTGCCGGAGATAG-3'. A gel imaging analysis system (Alpha Innotech ChemiImager 5500; BioSurplus, Inc., San Diego, CA, USA) was used to analyze the PCR results.

Determination of bone sialoprotein (BSP). Frozen tibia tissues (100 mg) were lysed in 1 ml ice-cold homogenization radioimmunopreciptation assay buffer (Wuhan Boster Biological Technology, Ltd., Wuhan, Boster) containing a protease inhibitor. The homogenates were centrifuged at 2,580 x g for 5 min at 4˚C and the supernatant was collected. The protein content was determined using a bicinchoninic acid assay (Beijing Tiandz Biological Technology Co., Ltd.), ensuring that each 20 μl contained 50 μg protein, and samples were stored at -20˚C. Each sample (6.08-6.44 μg) was separated using 10% sodium dodecyl sulfonate gel electrophoresis

Table I. BBB scores (n=8 per group).

Group	BBB score (days)			
	1	3	5	7
Normal	20.75±0.460	20.50±0.53	20.63±0.520	20.50±0.530
Sham + KRMB	20.63±0.520	20.75±0.46	20.50±0.530	20.38±0.520
Normal + KRMB	20.63±0.520	20.75±0.46	20.63±0.520	20.75±0.460
SCI + KRMB	0.000±0.000[a]	0.375±0.51[a]	0.750±0.463[a]	1.625±0.744[a]
SCI model	0.000±0.000[a]	0.250±0.46[a]	0.625±0.518[a]	1.500±0.756[a]

Data are presented as the mean ± standard deviation. [a]P<0.01 vs. normal group. BBB, Basso, Beattie and Bresnahan; KRMB, kidney reinforcing and marrow-beneficial medicine; SCI, spinal cord injury.

(60-120 V; 2 h) and transferred to a polyvinylidene difluoride membrane, then semi dry transfer membranes (both purchased from Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) were blocked with 5% calf serum albumin (Beyotime Biotechnology Co., Ltd., Shanghai, China) at room temperature for 1 h. Following this, the membranes were washed 3 times for 5 min with Tris-buffered saline with 0.05% Tween-20 (TBST) (Wuhan Boster Biological Technology, Ltd.). Then, the membranes were incubated overnight at 4˚C in blotting buffer containing a primary rabbit polyclonal antibody (1:300; BA2329; Wuhan Boster Biological Technology, Ltd.). Membranes were then washed using Tris-buffered saline (Wuhan Boster Biological Technology, Ltd.) and incubated for 1 h at room temperature in blotting buffer containing poly-horseradish peroxidase-conjugated streptavidin mouse anti-goat IgG antibody (1:3,000; bs-0294Ms; Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China). Membranes were then washed again with TBST for 5 min and a western blot was performed using 5-bromo-4-chloro-3-indolyl phosphate and p-nitroblue tetrazolium reagent (Beijing Tiandz Biological Technology Co., Ltd.) and β-actin (42 kD; Beijing Biosynthesis Biotechnology Co., Ltd., Beijing,China) was employed as the internal reference. Membranes were scanned using a Gel Imaging Analyzer (Six One Instrument Factory, Beijing, China), absorbance was measured using a D8 quasi dual beam UV-visible spectrophotometer [Runqee (Shanghai) Instrument Technology Co., Ltd., Shanghai, China] and the absorbance of protein bands was analyzed using gel analysis software (Image J; version 1.47; National Institutes of Health, Bethesda, MA, USA).

Statistical analysis. SPSS software, version 13.0 (SPSS, Inc., Chicago, IL, USA) was used to perform statistical analysis of the experimental data. Data are presented as the mean ± standard deviation. The electrophoresis results were determined using FluorChem software, version 2.0 (Gene Genus; Syngene, Frederick, MD, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

BBB score. During the experimental process, a total of 32 rats succumbed to mortality; 18 rats succumbed to mortality in the SCI model group, and 14 rats succumbed to mortality in the SCI + KRMB group, according to the experimental conditions of strict supplements. As presented in Table I, the BBB scores in the SCI and SCI + KRMB groups were significantly reduced in comparison to the normal group (P<0.01) at 1, 3, 5 and 7 days after the operation. These results suggest that the SCI model was successfully established.

BMD detection. As presented in Table II and Fig. 1, the expression of BMD was not significantly different among the groups at 1 or 2 weeks following the operation. However, BMD levels in the SCI model group were significantly lower than those in the normal group (P<0.01). Furthermore, BMD levels in the SCI group were significantly lower than that in the SCI + KRMB group at 6 (P<0.05), 8 and 10 weeks (P<0.01).

Serum BGP expression levels. As presented in Table III, the serum expression levels of BGP in the SCI model group were significantly higher than those in the normal, sham + KRMB (P<0.01) and normal + KRMB (P<0.05) groups at each time point, and significantly lower than the normal + KRMB group (P<0.05), and the normal and sham + KRMB group (P<0.01). The level of serum BGP in the SCI + KRMB group was significantly increased compared with the normal, sham + KRMB and normal + KRMB group at each time point (P<0.01).

Hepcidin mRNA expression. The primer internal reference gene and target genes of each group were subjected to RT-PCR amplification, which was performed using rat liver tissue. RT-PCR analysis revealed two bands at 200 and 263 bp (Fig. 2). As presented in Table IV, the image analysis software indicated that the expression of hepcidin mRNA in the normal, sham + KRMB and normal + KRMB group was significantly higher than that in the SCI + KRMB and SCI model groups at each time point (P<0.01). Hepcidin mRNA expression in the SCI + KRMB group was significantly higher than that in the SCI model group at 1, (P<0.05), 2, 4, 6, 8 and 10 weeks (P<0.01).

BSP expression in rat tibial bone tissue. As presented in Table V and Fig. 3, there are no statistically significant differences in BSP expression among the normal, SCI + KRMB and normal + KRMB groups. However, the BSP expression

Table II. BMD detection results (n=8 per group).

	BMD (g/cm^2; weeks)					
Group	1	2	4	6	8	10
Normal	0.210±0.010	0.209±0.010	0.211±0.009[a]	0.209±0.008[a]	0.208±0.009[a]	0.209±0.005[a]
Sham + KRMB	0.210±0.010	0.211±0.011	0.209±0.009[a]	0.211±0.009[a]	0.210±0.009[a]	0.210±0.013[a]
Normal + KRMB	0.210±0.009	0.209±0.011	0.208±0.013[a]	0.208±0.012[a]	0.210±0.011[a]	0.209±0.010[a]
SCI + KRMB	0.210±0.009	0.203±0.011	0.196±0.007	0.190±0.007[b]	0.188±0.007[a]	0.190±0.006[a]
SCI model	0.209±0.012	0.202±0.011	0.189±0.010	0.178±0.009	0.172±0.010	0.172±0.009

Data are presented as the mean ± standard deviation. [a]P<0.01 and [b]P<0.05 vs. SCI model group. BMD, bone mineral density; KRMB, kidney reinforcing and marrow-beneficial medicine; SCI, spinal cord injury.

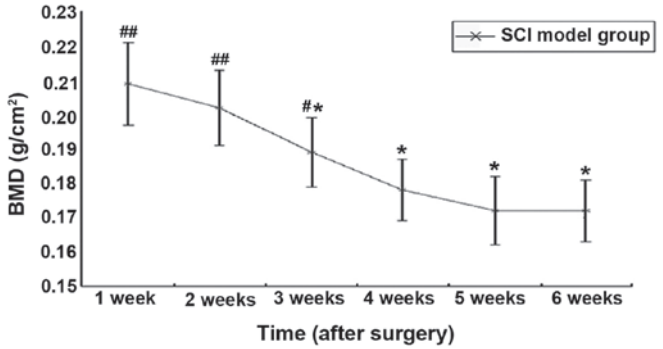

Figure 1. BMD levels following SCI. The BMD values at 2 weeks are significantly different to those at 4, 6 and 8 weeks (P<0.01), and the BMD value at 6 weeks is lower than that at 4 weeks (P<0.05). No statistically significant difference was observed between 6, 8 and 10 weeks. *P<0.05 vs. 2 weeks after surgery; #P<0.01, ##P<0.05 vs. 6 weeks after surgery. BMD, bone mineral density; SCI, spinal cord injury.

levels in the SCI + KRMB and SCI model groups were significantly higher compared with the normal, sham + KRMB and normal + KRMB groups at each time point (P<0.01). In addition, the expression of BSP in the SCI model group was higher than that in the SCI + KRMB group at 1 (P<0.05) 2, 4, 6, 8 and 10 weeks (P<0.01).

Discussion

According to the theory of traditional Chinese medicine, bone ingrowth relies on providing bone marrow with sufficient nutrition (5,6). The main pathogenesis of OP resulting from SCI is marrow deficiency and kidney asthenia, and the mechanism underlying KRMB Chinese medicine in treating OP is a current area of interest (6).

At present, a number of methods exist to establish the SCI model, including the spinal cord transection model that is commonly used in studies as a result of its simple operational procedure and light secondary reaction (13). The present study cut the T10 dura and spinal cord in rats, removed 2 mm spinal cord tissue from below the T10 spinal segment and filled the gap with gelfoam sponge. The advantage of using this surgical procedure is that it operates at the correct anatomical position and is consistent with the degree of injury. The operation results in motor and sensory function loss below the cross section, causing dysfunction that is attributed to the primary injury of the spinal cord and diminishing the risk of human error (14).

Characteristics of hind limb motor function were assessed in accordance with the BBB scale (15-17). At 1, 3, 5 and 7 days following the operation, the BBB scores of the SCI model and SCI + KRMB groups were significantly decreased compared with the normal group (P<0.01), suggesting that the SCI model was duplicated successfully.

BMD is a reliable criterion for evaluating the effect of drugs in treating OP, and is an important index to quantify bone mineralization in bone metabolism; therefore, it is regarded as the gold standard criteria for diagnosing OP (18). BMD typically decreases 1 week following SCI, a reduction in bone mass appears at 2 weeks and marked OP appears at 4 weeks, reaching its peak and flattening at 6 weeks (19). In the present study, the BMD levels in the SCI model group were significantly lower compared with the SCI + KRMB group at 6 (P<0.05), 8 and 10 weeks (P<0.01). These results indicate that KRMB increases BMD in rats with OP following SCI.

A preliminary study of bone metabolism following SCI demonstrated that osteoclastic resorption performance, with or without slight enhancement of bone formation, is the primary cause of the high-turn-over OP (20), and that an increase in the expression of BGP indicates bone formation (21). In the current study, the expression levels of serum BGP in the SCI model group were significantly higher than those in the normal group (P<0.01), and that, consistent with the literature (22), this was associated with mild bone formation enhancement following SCI. The measurement of serum BGP is used in evaluating the effect and efficacy of treatments for OP (23,24). Furthermore, the expression levels of serum BGP in the SCI + KRMB group were significantly higher compared with the normal group at each time point (P<0.01). Therefore, KRMB may be able to upregulate the expression of serum BGP.

The association between iron metabolism and OP is being increasingly recognized, and both clinical and experimental studies suggest that an iron overload may be a risk factor for OP (24,25). In addition, a previous study has demonstrated that hepcidin expression in OP model groups is significantly different compared with control groups (26). Hepcidin can significantly decrease the apoptosis rate of human fetal OB 1.19 cells and enhance their calcification (27). These

Table III. BGP levels in rat serum.

	Serum BGP (pg/ml; weeks)					
Group	1	2	4	6	8	10
Normal	96.67±5.90[a]	96.74±7.96[a]	96.31±6.50[a]	94.77±6.35[a]	96.13±6.63[a]	97.50±6.34[a]
Sham + KRMB	101.20±5.88[a]	99.16±6.98[a]	101.85±6.25[a]	96.49±5.71[a]	97.90±6.49[a]	98.88±5.47[a]
Normal + KRMB	97.84±6.32[a]	98.35±9.05[a]	100.83±6.01[a]	96.87±5.51[a]	97.03±4.55[a]	98.51±6.36[a]
SCI + KRMB	131.93±7.80[b-d]	138.88±4.43[b-d]	142.08±7.16[b-d]	162.50±7.31[b-d]	160.40±10.13[b-d]	156.81±6.90[b-d]
SCI model	124.79±4.81[b-e]	123.23±4.80[b-e]	120.65±4.60[b-e]	115.76±5.06[b-e]	112.97±5.17[b-e]	112.45±4.70[b-e]

Data are presented as the mean ± standard deviation. [a]P<0.01 vs. SCI + KRMB group; [b]P<0.01 vs. normal group; [c]P<0.01 vs. sham + KRMB group; [d]P<0.05 vs. normal + KRMB; [e]P<0.05 vs. SCI + KRMB group. BGP, bone gamma-carboxyglutamic-acid; KRMB, kidney reinforcing and marrow-beneficial medicine; SCI, spinal cord injury.

Table IV. Hepcidin mRNA expression in rat liver tissue (n=5 per group).

	Optical density (Hepcidin/β-actin; weeks)					
Group	1	2	4	6	8	10
Normal	0.621±0.030[a,b]	0.620±0.031[a,b]	0.620±0.038[a,b]	0.622±0.032[a,b]	0.623±0.039[a,b]	0.618±0.033[a,b]
Sham + KRMB	0.609±0.018[a,b]	0.608±0.020[a,b]	0.613±0.027[a,b]	0.616±0.029[a,b]	0.621±0.021[a,b]	0.627±0.019[a,b]
Normal + KRMB	0.614±0.017[a,b]	0.617±0.045[a,b]	0.619±0.039[a,b]	0.622±0.039[a,b]	0.628±0.020[a,b]	0.641±0.011[a,b]
SCI + KRMB	0.370±0.017	0.387±0.023	0.415±0.021	0.428±0.020	0.429±0.033	0.444±0.033
SCI model	0.341±0.015[c]	0.323±0.018[a]	0.313±0.016[a]	0.300±0.011[a]	0.299±0.010[a]	0.293±0.018[a]

Data are presented as the mean ± standard deviation. [a]P<0.01 vs. SCI + KRMB group; [b]P<0.01 vs. SCI model; [c]P<0.05 vs. SCI + KRMB. KRMB, kidney reinforcing and marrow-beneficial medicine; SCI, spinal cord injury.

Table V. BSP expression in rat tibial bone tissue (n=5 per group).

	Grey level ratio (BSP/β-actin; weeks)					
Group	1	2	4	6	8	10
Normal	0.223±0.017[a,b]	0.227±0.010[a,b]	0.229±0.009[a,b]	0.224±0.012[a,b]	0.231±0.011[a,b]	0.230±0.009[a,b]
Sham + KRMB	0.229±0.008[a,b]	0.226±0.009[a,b]	0.238±0.011[a,b]	0.228±0.010[a,b]	0.225±0.008[a,b]	0.227±0.013[a,b]
Normal + KRMB	0.234±0.013[a,b]	0.232±0.010[a,b]	0.228±0.013[a,b]	0.225±0.009[a,b]	0.223±0.007[a,b]	0.223±0.004[a,b]
SCI + KRMB	0.320±0.014	0.313±0.008	0.306±0.014	0.281±0.012	0.281±0.016	0.282±0.009
SCI model	0.339±0.009[c]	0.343±0.013[a]	0.372±0.019[a]	0.399±0.018[a]	0.397±0.015[a]	0.388±0.025[a]

Data are presented as the mean ± standard deviation. [a]P<0.01 vs. SCI + KRMB group; [b]P<0.01 vs. SCI model; [c]P<0.05 vs. SCI + KRMB. BSP, bone sialoprotein; KRMB, kidney reinforcing and marrow-beneficial medicine; SCI, spinal cord injury.

studies suggest that hepcidin may have a correlation with OB cell metabolism. In the present study, hepcidin expression in the SCI model group was reduced compared with the SCI + KRMB group at 1 (P<0.05), 2, 4, 6, 8 and 10 weeks (P<0.01). This result suggests that KRMB Chinese medicine is able to increase the expression of hepcidin mRNA in rat livers, which may be involved in the development of OP following SCI.

BSP is the predominant non-collagen material in bone extracellular matrix that participates in cell adhesion, transfer and signal identification associated with the formation of bone tissue and alteration (28). The expression of BSP serves a crucial function in the process of bone absorption (29,30). BSP expression in the SCI model group was significantly higher than that in the normal group at each time point (P<0.01), and was increased compared with the SCI + KRMB group at 1 (P<0.05), 2, 4, 6, 8 and 10 weeks (P<0.01). This suggests that the expression of BSP in the early stage of SCI in rats may be involved in activating the process of bone resorption. Therefore, it may be suggested that KRMB Chinese medicine

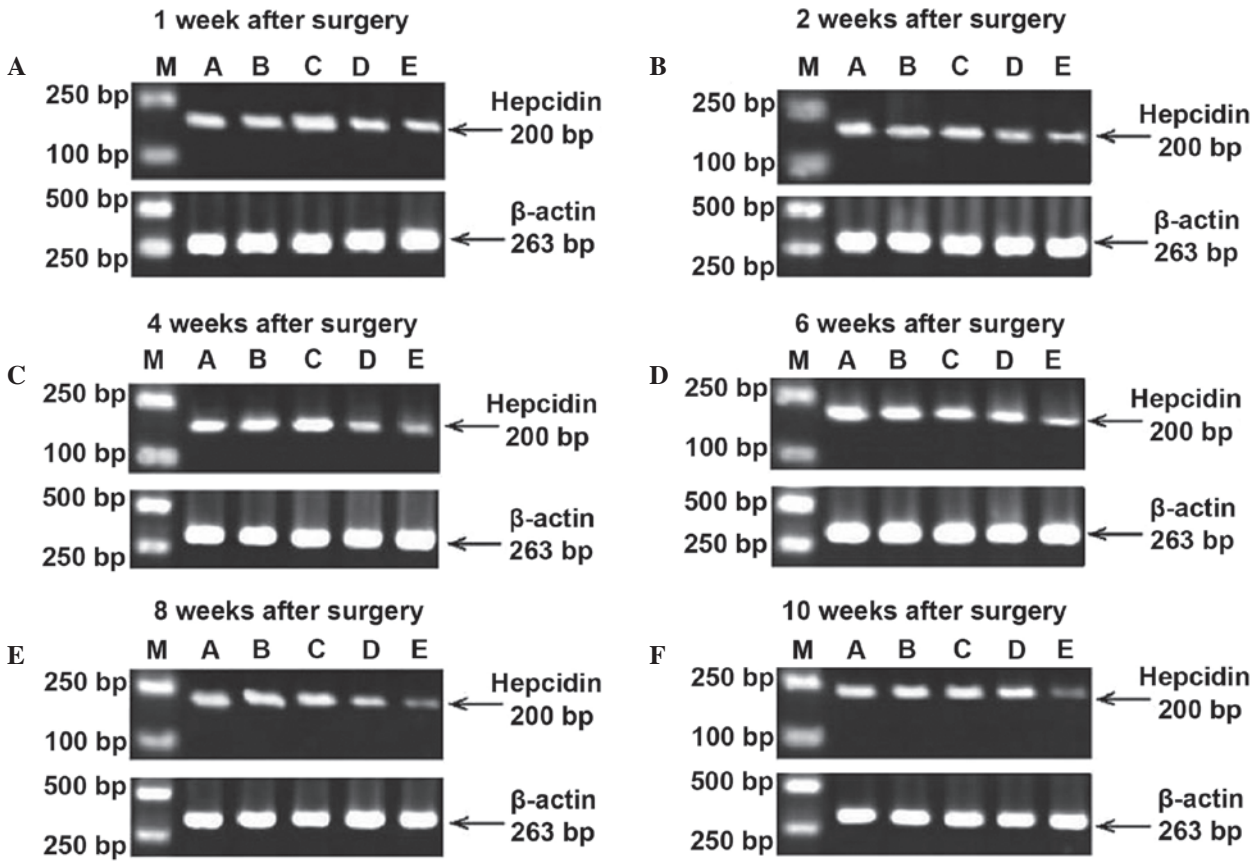

Figure 2. Gel images from reverse transcription polymerase chain reaction amplification of hepcidin mRNA expression at (A) 1, (B) 2, (C) 4, (D) 6, (E) 8 and (F) 10 weeks after operation. m, DNA marker; a, normal group; b, sham operation + KRMB group; c, normal + KRMB group; d, SCI + KRMB group; e, SCI model group. KRMB, kidney reinforcing and marrow-beneficial medicine; SCI, spinal cord injury.

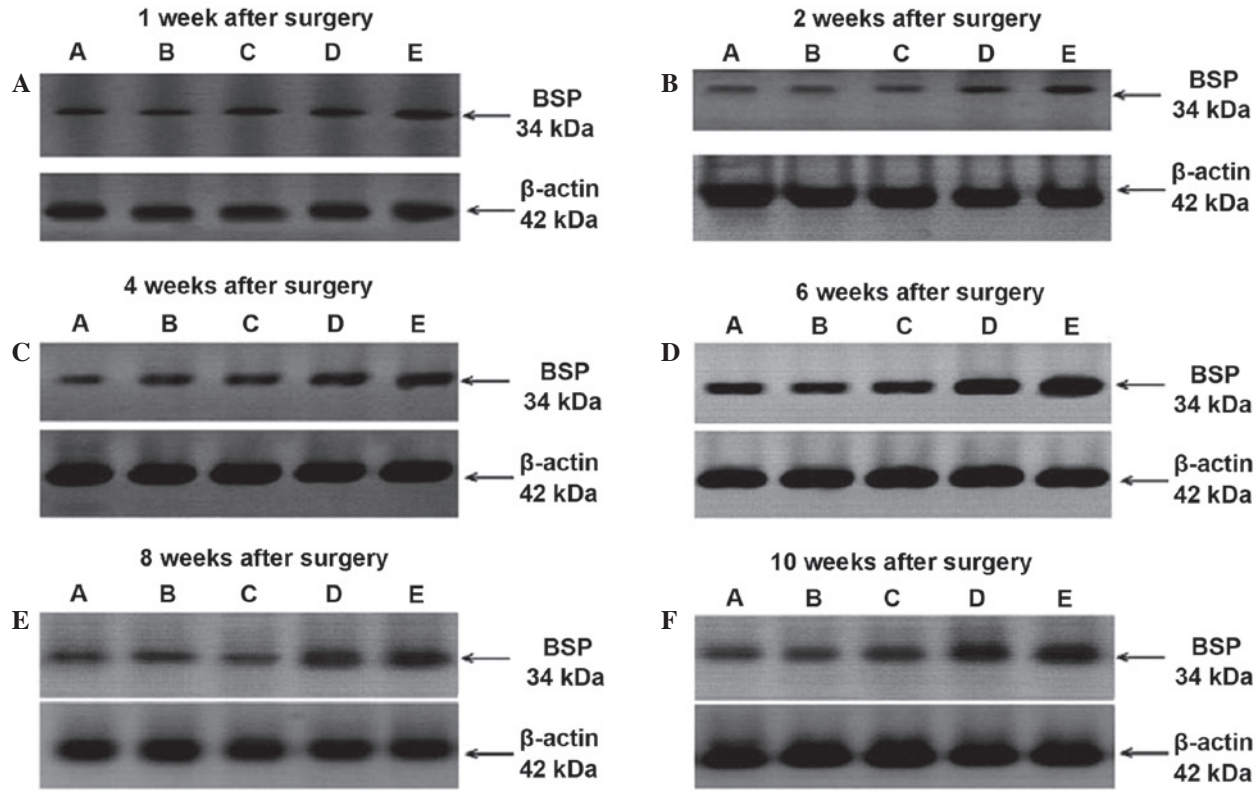

Figure 3. Western blots of BSP expression at (A) 1, (B) 2, (C) 4, (D) 6, (E) 8 and (F) 10 weeks after operation. A, normal group; B, sham operation + KRMB group; C, normal + KRMB group; D, SCI + KRMB group; E, SCI model group. BSP, bone sialoprotein; KRMB, kidney reinforcing and marrow-beneficial medicine; SCI, spinal cord injury.

delays the progression of OP following SCI by reducing the expression of BSP in tibia bone tissue in rats, resulting in the reduction of bone resorption.

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