ORIGINAL ARTICLE

Effect of Huogu Ⅱ Formula (活骨Ⅱ方) with Medicinal Guide *Radix Achyranthis Bidentatae* on Bone Marrow Stem Cells Directional Homing to Necrosis Area after Osteonecrosis of the Femoral Head in Rabbit*

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ABSTRACT **Objective**: To investigate the effect of Huogu Ⅱ Formula (活骨Ⅱ方) with medicinal guide *Radix Achyranthis Bidentatae* (Ach) on bone marrow stem cells (BMSCs) homing to necrosis area after osteonecrosis of the femoral head (ONFH) frozen by liquid nitrogen in rabbit as well as to explore the mechanism of prevention and treatment for ONFH. **Methods**: The animal model of ONFH was established by liquid nitrogen frozen on the rabbit left hind leg. Forty-eight Japanese White rabbits were randomly assigned to sham-operated group, model group, Huogu Ⅱ group, and Huogu Ⅱ plus Ach group, with 12 rabbits in each. During the course of ONFH animal model establishment, all rabbits were subcutaneously injected with recombinant human granulocyte colony-stimulating factor [rhG-CSF, 30 μg/(kg·day) for continuous 7 days]. Meanwhile, normal saline and decoction of the two formulae were administrated by gavage, respectively. White blood cells (WBC) were counted in peripheral blood before and after injection of rhG-CSF. Materials were drawn on the 2nd and 4th weeks after model built; bone glutamine protein (BGP) and bone morphogenetic protein 2 (BMP2) levels in serum were tested. Histopathologic changes were observed by hematoxylin and eosin (HE) staining. BMP2 mRNA levels were detected with *in situ* hybridization (ISH) staining. 5-Bromo-2'-deoxyuridine (BrdU) and stromal cell derived factor 1 (SDF-1) were measured by immunohistochemical assay in femoral head of the left hind leg. **Results**: Compared with the sham-operated group, the ratio of empty lacuna, serum BGP, and SDF-1 level in the model group increased significantly, and BMP2 in both serum and femoral head decreased significantly. However, in comparison with the model group, the empty lacuna ratio of Huogu Ⅱ group and Huogu Ⅱ plus Ach group decreased obviously in addition to the levels of serum BGP and BMP2, and the expressions of BMP2 mRNA, BrdU, and SDF-1 increased significantly. Above changes were particularly obvious in Huogu Ⅱ plus Ach group. BGP and SDF-1 on the 2nd week and empty lacuna rate and serum BMP2 level on the 4th week in Huogu Ⅱ group significantly exceeded their counterparts. On the 2nd week, only in Huogu Ⅱ plus Ach group that the BrdU counting rose significantly. On the 4th week, empty lacuna rate and serum BMP2 level in Huogu Ⅱ plus Ach group exceeded those in Huogu Ⅱ group distinctively. **Conclusions**: To a certain extent, the medicinal guide Ach improves the preventive and therapeutic effects of Huogu Ⅱ Formula on experimental ONFH model. The possible mechanism of this is related to its promoting effect on directional homing of BMSCs to the necrosis area.

KEYWORDS osteonecrosis of the femoral head, bone marrow stem cells, homing, guide drug to affected area

To date, an ideal therapy with satisfactory effect for osteonecrosis of the femoral head (ONFH) still has not been found in the medical community. Repairing the injured tissue with bone marrow stem cells (BMSCs) is a new exploration in the application of stem cells.[1] Previous studies indicate that BMSCs could be summoned by inflammatory irritation of lesion focus and thus homing to the site. However, its effect is extremely limited.[2] Thus, it has been attracting increasing attention in recent years on devising an agent or a method, which can be able to promote the process of BMSCs homing to pathologic tissues.

The Huogu Ⅱ Formula (活骨Ⅱ方) is one of our frequently used empirical recipes in treating ONFH.[3-5]

*Supported by the National Natural Science Foundation of China (No. 30672770)
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DOI: 10.1007/s11655-011-0940-7

Chin J Integr Med 2012 Oct;18(10):761-768

In clinic, various medicinal guides are added to it according to a specific situation of different patients for better curative effects. Since medicinal guide has obvious effect-directing function, whether there is a link between adoption of medicinal guide and homing of BMSCs has been a riddle for us to solve. This study is designed to employ granulocyte colony-stimulating factor (G-CSF) to mobilize BMSCs to peripheral blood and then observe the influence of Huogu Ⅱ Formula plus *Radix Achyranthis Bidentatae* (Ach), a medicinal guide, on mobilized BMSCs homing to necrosis area of ONFH.

METHODS

Animals

Forty-eight adult male Japanese White rabbits with body weight of 2.2 to 2.8 kg were provided by Beijing Merial Vital Laboratory Animal Technology Co., Ltd. (certificate No. SYXK1100-0022). The animals received a standard laboratory diet and water *ad libitum*. The experimental protocol was approved by the Animal Experiment Ethics Committee of the China Academy of Chinese Medical Sciences.

Drugs and Reagents

The Huogu Ⅱ Formula (consists of *Radix Astragali*, *Angelica Sinensis*, *Radix Paeonia Rubra*, *Rhizoma Ligustici Chuanxiong*, *Lumbricus*, *Ramulus Cinnamoni*, etc.) and Ach, provided by the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, were decocted into solution, which contained crude drug in 1 g/mL. The recombinant human G-CSF (rhG-CSF, Lot No. 20080503) was purchased from Shandong GeneLeuk Biopharmaceutical Co., Ltd., China. 5-Bromo-2'-deoxyuridine (BrdU) was provided by Sigma-Aldrich Co., Ltd., USA. Mouse anti-BrdU was supplied by Zymed Laboratories, Inc., USA. Bone glutamine protein (BGP) radioimmunoassay (RIA) kit (Lot No. 20090120) was provided by the Institute of Radioimmunity of Scientific Developing Center of PLA General Hospital, China. Bone morphogenetic protein 2 (BMP2) enzyme-linked immunospecific assay (ELISA) kit (Lot No. 20090301) were provided by USCN Life Science Co., Ltd., China. BMP2 *in situ* hybridization (ISH) detection kit (Lot No. 20090311), anti-stromal cell derived factor 1 (SDF-1), and strept avidein-biotin complex (SABC) detection kit were supplied by Wuhan Boster Bio-engineering Ltd. Co., China.

Grouping and Treatment

Forty-eight healthy Japanese White rabbits were divided randomly into 4 groups (each was composed of 12 rabbits) as sham-operated group, model group, Huogu Ⅱ group, and Huogu Ⅱ plus Ach group. Except for the sham-operated group, the animal model of ONFH was established by liquid nitrogen freezing method as described previously[6] for the other 3 groups. Briefly, 3% pentobarbital sodium was injected in a dose of 30 mg/kg via ear vein to anesthetize the rabbit, then cut the skin, subcutaneous tissue, and deep fascia open, dissect directly to the capsule of hip joint, open the capsule with T-shaped incision, retain the round ligament, dislocate the femur head, and cover the tissue around femoral head with dry gauze for protection. The femoral head was frozen for 3 min with the gauze soaked with liquid nitrogen. Then, the femoral head was re-warmed with normal saline and the hip joint was relocated. Finally, the incision was sutured. For the sham-operated group, the femoral head was only exposed without frozen. All model animals were subcutaneously injected with rhG-CSF in a dose of 30 μg/(kg•day) for 7 days. Rabbits of Huogu Ⅱ group and Huogu Ⅱ plus Ach group were administrated with Huogu Ⅱ decoction and Huogu Ⅱ plus Ach (15 g each dose) decoction in a dose of 6 g/(kg•day) by gavage, respectively, for 4 weeks. Meanwhile, the other groups were administrated with normal saline in the same volume. All animals were injected intramuscularly with 200 000 U penicillin for 7 days to prevent infection. To label BMSCs, all rabbits were intraperitoneally injected with BrdU (50 mg/kg) for the successive 3 days before sampling.

Count of the Peripheral White Blood Cells

Before (0 day) and after rhG-CSF injection 3, 5, and 7 days, 20 μL blood was taken from auricular vein, respectively. White blood cells (WBC) in peripheral blood were tested by automatic biochemical analyzer at different time points as mentioned above.

Levels of Serum BGP and BMP2

Blood samples were collected by heart puncture at the end of the 2nd and 4th weeks, respectively, and then serum was separated and preserved at –20 ℃. BGP level was measured by RIA according to the standard procedure of the kit. Finally, BMP2 level in serum was detected in accordance with the instructions on ELISA kit.

Pathomorphological Observation

At the end of the 2nd and 4th weeks of this

experiment, 4 rabbits of each group were sacrificed to obtain femoral heads of left hind legs followed by fixation in 4% paraformaldehyde and embedding in paraffin. Then, slices were done to make slides 5 μm in thickness, and hematoxylin and eosin (HE) staining was carried out. Five visions were chosen randomly under high-power light microscope and 50 lacunae in each vision field were captured to calculate the empty lacunae rate. According to the previous reports,[7-9] osteonecrosis was defined as the presence of all three of the following criteria: diffuse presence of empty lacunae, pyknotic nuclei of ghost osteocytes in the bone trabeculae, and necrosis of the adjacent marrow and stromal elements.

Expression of BMP2 mRNA in Femoral Head

After dewaxing, the sections were treated in 3% peroxide oxygen and incubated in proteinase K followed by 3 washes with phosphate buffered saline (PBS). Pre-hybridization and hybridization steps were carried out at 38 to 42 ℃ for 3 and 15 h, respectively. After post-hybridization washing, the sections were incubated with primary antibody and then with second antibody, and histochemical detection was performed using diamino-benzidine (DAB). PBS was utilized instead of probe as negative control. The expression of mRNA was observed under microscope, and images were collected under 200× microscopic magnification. To be more specific, 3 fields of vision were selected for each specimen. Finally, SPOT MIS, an image analysis software, was applied to perform positive cell counting in each field.

Expressions of BrdU and SDF-1 in Femoral Head

For BrdU detection, the steps were as follows: paraffin section was dewaxed and rehydrated, incubated in 1 mol/L hydrochloride at 40 ℃ for 1 h, washed by PBS and digested by 0.04% pancreatin at 40 ℃ for 20 min to destruct the DNA structure, treated by 3% peroxide oxygen and then digested by compound enzyme at 37 ℃, then washed by PBS, blocked by serum, added with primary antibody, and then kept under 4 ℃ overnight. SABC detection kit was employed to detect the BrdU expression in femoral head. For the detection of SDF-1, after rehydration, it was treated by 3% peroxide oxygen directly and the following steps were same as those of BrdU. SPOT MIS system was utilized to perform positive cell counting and grayscale analysis in visual field under 200× magnification.

Statistical Analysis

The data were presented as mean ± standard deviation. One-way ANOVA was performed for comparison between groups. All statistical analyses were performed using SPSS 13.0 and *P* values less than 0.05 were considered to be significantly different.

RESULTS

Mobilization of BMSCs

After rhG-GSF injection, WBC count in peripheral blood increased remarkably (Figure 1), and as time went on, WBC count further increased until reaching its peak on the 7th day. There was no statistical significance among the 4 groups, and all these phenomena mentioned above suggested that the application of rhG-CSF was effective in mobilizing BMSCs to peripheral blood.

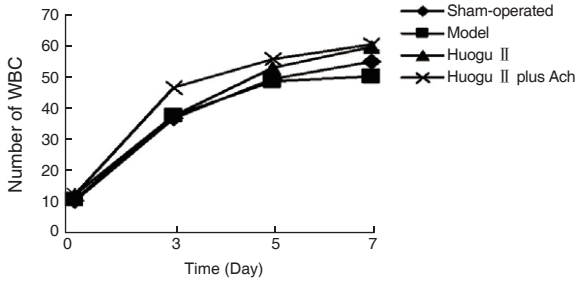

Figure 1. Comparison of WBC Count among Groups before and after rhG-CSF Injection

Pathologic Changes of Femoral Head

Two weeks after freezing by liquid nitrogen, the trabeculae became thinner, partially broken, and underwent necrosis. Karyopyknosis and nuclear margination could be observed in osteocyte (Figure 2A). In addition, the empty lacunae rate reached 38.2% (Figure 2B). Four weeks after the experiment, the following phenomena were observed: decreased osteoblasts and increased osteoclasts in the surrounding regions of trabeculae. Necrosis area and empty lacunae rate further grew in number (*P*<0.01). There were diminished bone marrow cells inside the cavity and increased necrotic cells. The proliferation and hypertrophia of adipocytes and distinct fibrosis were found. However, in Huogu Ⅱ group and Huogu Ⅱ plus Ach group, trabeculae were more compact. Abundant osteoblasts and a few osteoclasts were observed around trabeculae. The nuclear of osteocyte was plump and empty lacuna ratio was lower than that of the model group (Figure 2B, *P*<0.01). Ample bone marrow cells were found in cavity, and no obvious sign of proliferation and hypertrophia could be

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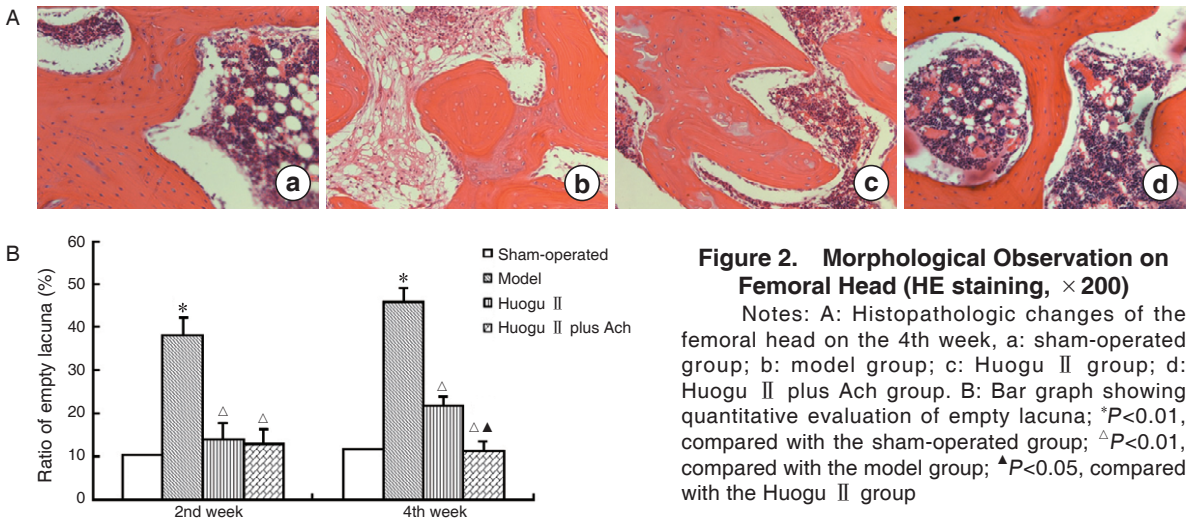

Figure 2. Morphological Observation on Femoral Head (HE staining, × 200)

Notes: A: Histopathologic changes of the femoral head on the 4th week, a: sham-operated group; b: model group; c: Huogu Ⅱ group; d: Huogu Ⅱ plus Ach group. B: Bar graph showing quantitative evaluation of empty lacuna; $^{*}P<0.01$, compared with the sham-operated group; $^{\triangle}P<0.01$, compared with the model group; $^{\blacktriangle}P<0.05$, compared with the Huogu Ⅱ group

observed among fat cells. There were abundant blood vessels with increased vascular area. Especially on the 4th week of the experiment, empty lacunae rate of Huogu Ⅱ plus Ach group was significantly lower than that of Huogu Ⅱ group and was roughly equal to the data of sham-operated group (Figure 2B, $P<0.01$).

BGP and BMP2 Levels in Serum

The level of BGP in model group exceeded that of sham-operated group drastically both at the end of the 2nd and 4th week ($P<0.05$ or $P<0.01$, Figure 3). Compared with the model group, BGP levels of Huogu Ⅱ group and Huogu Ⅱ plus Ach group were obviously higher at both time points ($P<0.01$ and $P<0.05$, respectively). Besides, BGP level of Huogu Ⅱ plus Ach group was higher than that of Huogu Ⅱ group on the 2nd week ($P<0.05$, Figure 3).

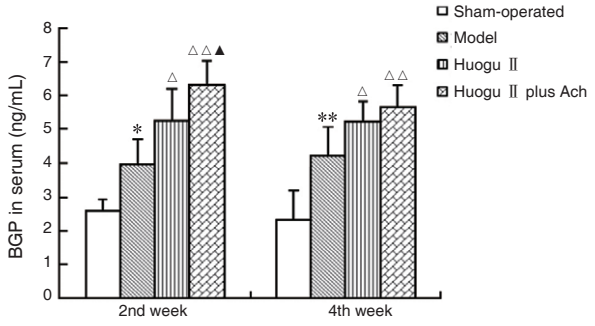

Figure 3. Comparison of Serum Level of BGP by RIA

Notes: $^{*}P<0.05$, $^{**}P<0.01$, compared with sham-operated group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$, compared with the model group; $^{\blacktriangle}P<0.05$, compared with the Huogu Ⅱ group

On the 2nd week, BMP2 level of the model group was similar to that of sham-operated group but decreased markedly on the 4th week (Figure 4, $P<0.01$). However, in the Huogu Ⅱ group and Huogu Ⅱ plus Ach group, BMP2 levels obviously increased compared with that of model group ($P<0.01$) both on the 2nd and 4th weeks. On the 4th week, BMP2 level of Huogu Ⅱ plus Ach group was higher than that of Huogu Ⅱ group, and the difference was of statistical significance ($P<0.05$, Figure 4).

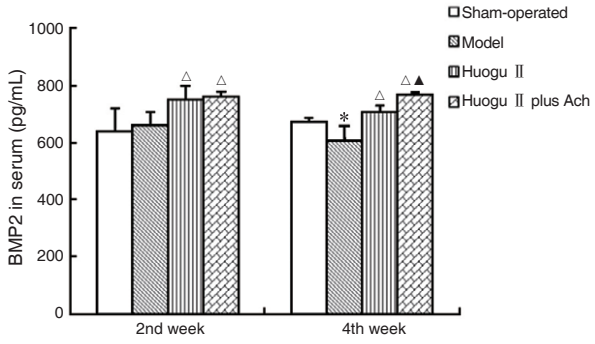

Figure 4. Comparison of Serum Level of BMP2 by ELISA

Notes: $^{*}P<0.01$, compared with sham-operated group; $^{\triangle}P<0.01$, compared with model group; $^{\blacktriangle}P<0.05$, compared with the Huogu Ⅱ group

BMP2 mRNA Expression in Femoral Head

Compared with the model group, BMP2-positive cells in the Huogu Ⅱ group and Huogu Ⅱ plus Ach group showed an increasing trend on the 2nd week. However, the comparison results among these groups did not possess statistical significance (Figure 5). On the 4th week, the number of BMP2-positive cells in model group was lower than that of sham-operated group clearly ($P<0.05$). In contrast with the model group, the numbers of BMP2-positive cells of Huogu Ⅱ group and Huogu Ⅱ plus Ach group rose greatly ($P<0.01$).

Expressions of BrdU and SDF-1 in Femoral Head

Compared with the sham-operated group, the number of BrdU-positive cells in the model group

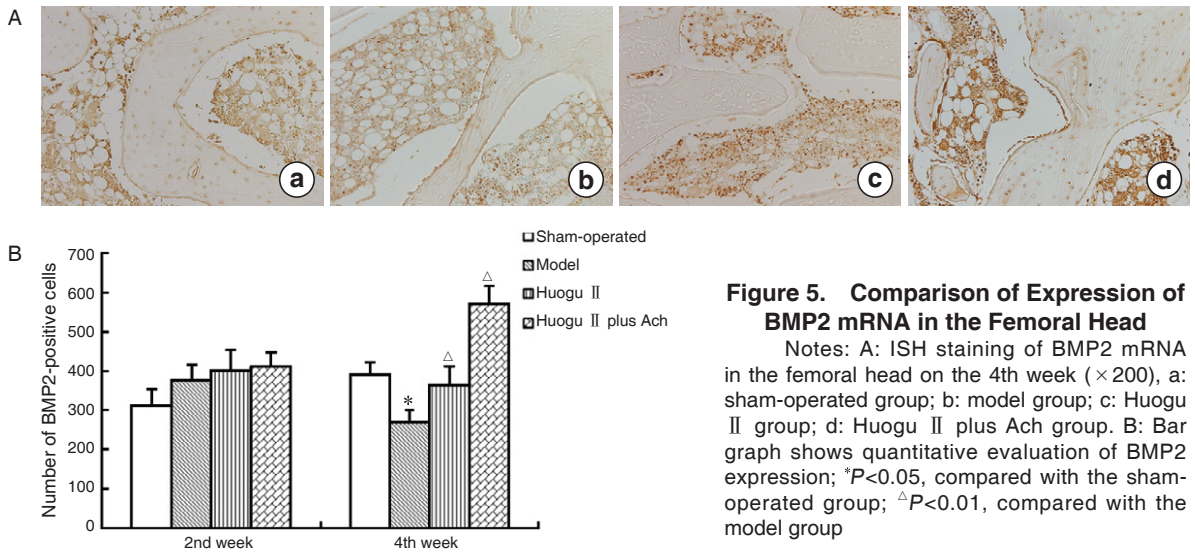

Figure 5. Comparison of Expression of BMP2 mRNA in the Femoral Head

Notes: A: ISH staining of BMP2 mRNA in the femoral head on the 4th week (×200), a: sham-operated group; b: model group; c: Huogu Ⅱ group; d: Huogu Ⅱ plus Ach group. B: Bar graph shows quantitative evaluation of BMP2 expression; $^{*}P<0.05$, compared with the sham-operated group; $^{\triangle}P<0.01$, compared with the model group

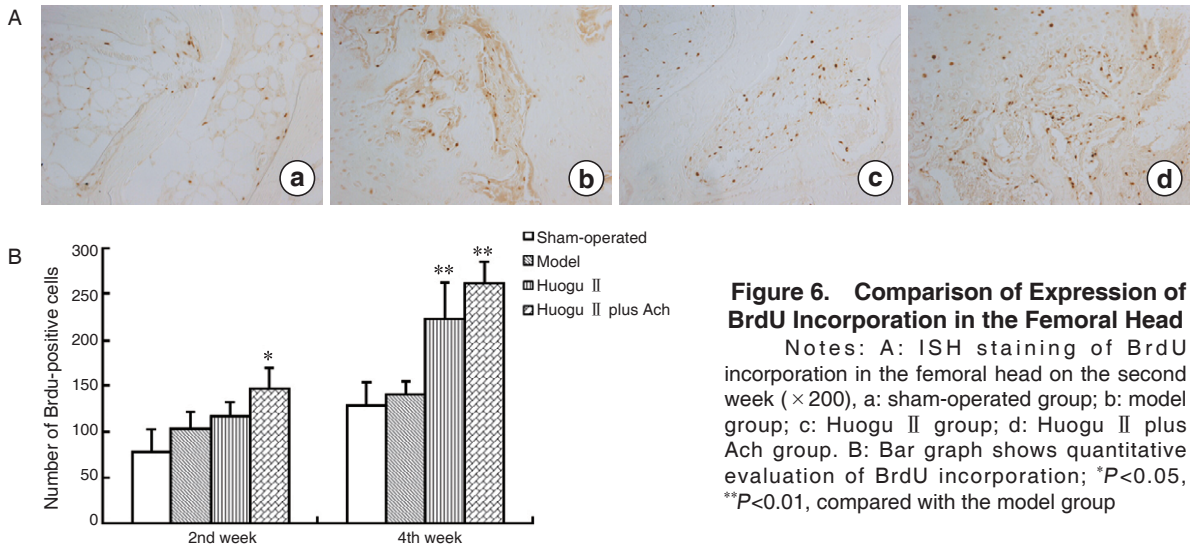

Figure 6. Comparison of Expression of BrdU Incorporation in the Femoral Head

Notes: A: ISH staining of BrdU incorporation in the femoral head on the second week (×200), a: sham-operated group; b: model group; c: Huogu Ⅱ group; d: Huogu Ⅱ plus Ach group. B: Bar graph shows quantitative evaluation of BrdU incorporation; $^{*}P<0.05$, $^{**}P<0.01$, compared with the model group

increased both on the 2nd and 4th week, but there were no statistical difference (Figure 6, $P>0.05$). Compared with the model group, on the 2nd week, only the positive cells of Huogu Ⅱ plus Ach group ascent significantly ($P<0.05$), while on the 4th week the positive cells of both Huogu Ⅱ group and Huogu Ⅱ plus Ach group ascent drastically ($P<0.01$, Figure 6).

Integrated optical density (IOD) of positive SDF-1 in the model group increased sharply compared with the sham-operated group on the 2nd week (Figure 7, $P<0.01$). However, it dropped slightly, which was of no significant difference on the 4th week. Compared with the model group, IOD of SDF-1 in both Huogu Ⅱ group and Huogu Ⅱ plus Ach group underwent a drastic rise on the 2nd and 4th weeks ($P<0.01$). However, only on the 2nd week that IOD of Huogu Ⅱ plus Ach group was higher than that of Huogu Ⅱ group with a statistical significance ($P<0.05$, Figure 7).

DISCUSSION

To our knowledge, although application of Chinese medicine in treating early to middlestage ONFH tends to receive well effects, things generally become quite different when dealing with rapid progression cases, large necrosis area cases, and necrosis lesion nears weight-bearing area cases, etc., due to its slowness in taking effects. Thus, seeking the therapy of high efficiency and short-onset time is now a challenging task in clinic. Recently, mobilizing autologous BMSCs to peripheral blood and inducing them homing to necrosis focus to repair pathologic tissue is of potential value in clinical application. BMSCs homing refers to a course that, under the influence of various factors, autologous or exogenous BMSCs directionally and chemotacticly

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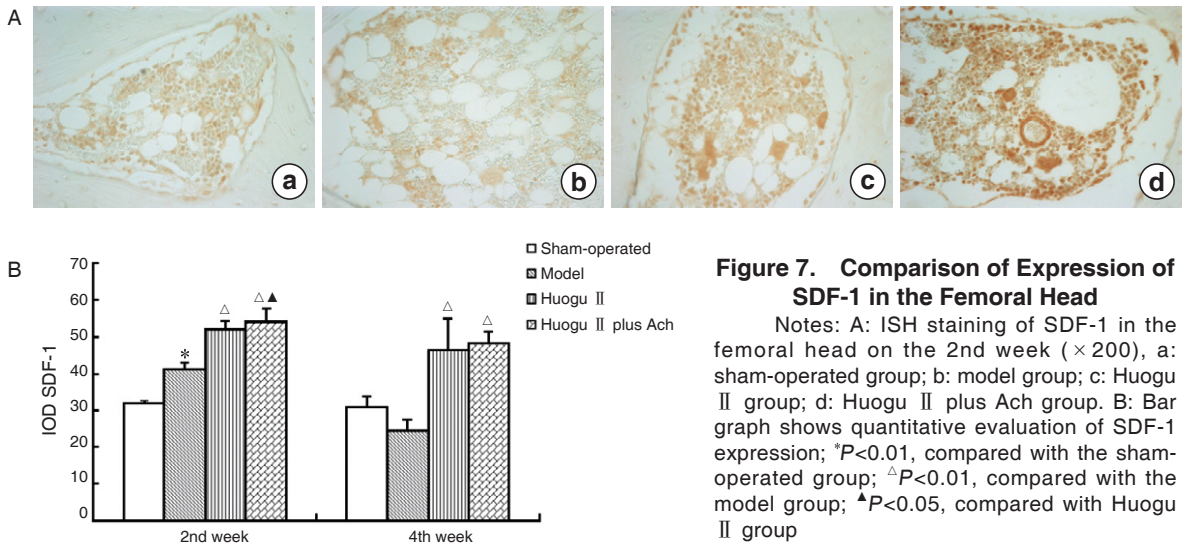

Figure 7. Comparison of Expression of SDF-1 in the Femoral Head

Notes: A: ISH staining of SDF-1 in the femoral head on the 2nd week (×200), a: sham-operated group; b: model group; c: Huogu Ⅱ group; d: Huogu Ⅱ plus Ach group. B: Bar graph shows quantitative evaluation of SDF-1 expression; $^{*}P<0.01$, compared with the sham-operated group; $^{\triangle}P<0.01$, compared with the model group; $^{\blacktriangle}P<0.05$, compared with Huogu Ⅱ group

migrate toward the target tissue. Afterwards, they are permanently planted there. Summoned by the inflammatory mediators and other substances, BMSCs home to the ischemic area and differentiate into local tissue cells or blood vessels to repair the injured tissue. However, the homing BMSCs are far less for all pathologic tissues and their repair effect is consequently too limited.[10,11] Thus, finding agents or methods that could promote BMSCs homing to lesions has been a hotspot for scholars specialized in ONFH at present.

Huogu Ⅱ Formula, one of our experienced prescriptions in treating ONFH, is modified from Buyang Huanwu Decoction (补阳还五汤) and is of qi-tonifying and blood-activating, meridian-dredging, and impediment-diffusing effects. Modern pharmacological studies show that *Radix Astragali* are effective in promoting the differentiation and proliferation of osteoblasts *in vitro*.[12,13] *Lumbricus* plays a role in osteocyte proliferation and differentiation in addition to matrix mineralization. It suppresses experimental alveolar bone resorption and facilitates its reconstruction by promoting osteogenesis.[14] Ligustrazine could prevent and control bone mass loss.[15] In previous studies, we found that Huogu Ⅱ Formula possesses positive effects on alleviating patients' symptoms and postponing the development of this disease.[16] For these patients who do not receive satisfactory curative effects, formula added with the medicinal guide commonly yields more favorable effects.[17] Take into account that stem cells play a vital role in bone repair and reconstruction as well as the medicinal guide could conduct other drugs reaching the lesion directly, we speculate that, to a certain extent, the medicinal guide possesses effects on promoting directional homing of BMSCs and it might be the reason that leads to its effects-enhancing effect. To explore the specific mechanism, we conduct this research to observe the influence of Huogu Ⅱ Formula and Huogu Ⅱ plus Ach—a medicinal guide that could enter the blood aspect and conduct other drugs downward to disease location—on BMSCs homing to lesions of ONFH.

The results of our experiment indicate that both Huogu Ⅱ Formula and Huogu Ⅱ plus Ach could manage ONFH. Specifically, they dropped the empty lacuna rate and reduced bone marrow fat conversion and cell necrosis. The condition of Huogu Ⅱ Formula plus Ach group improved more favorably and it is approximate to the manifestation of sham-operated group. Two weeks after freezing by liquid nitrogen, BMP2 and BGP levels were both elevated, suggesting that compensatory repair reaction functioned in the early stage of injury. BMP level dropped markedly at week 4, which indicated that the model animals were in decompensation stage. After administration with Huogu Ⅱ Formula and Huogu Ⅱ plus Ach, BMP2 and BGP levels increased significantly. Moreover, the decoction with Ach yields more beneficial effects. That tendency is consistent with our findings in histopathologic study.

In the experiment, after injection with G-CSF, WBC count of peripheral blood of these animals all increased by 7 times, which meant that BMSCs were activated successfully. However, the difference

among groups was of no statistical significance, which demonstrated that Chinese medicine administration did not distinctively influence the mobilizing effect of G-CSF. BrdU is a kind of analogues of thymidine, which is able to incorporate into the nucleus of cells in proliferation or division. Thus, it is widely used to label stem cell. After intraperitoneally injected with BrdU, an increasing trend of BrdU-positive expression was detected at week 2 and 4 in the ONFH model induced by liquid nitrogen through immunohistochemical method. It suggested that, after necrosis of competent cells (such as osteocytes, chondrocytes, and bone marrow cells), self-repair mechanism was initiated, afterwards, the activated stem cells joined in the repairing activity of bone. Although the BrdU-positive expression of model group was higher than the sham-operated group, the difference was of no statistical significance because homing stem cells were inadequate for sufficient repair. The histopathologic examination was featured by tissue necrosis (Figure 2Ab). After administration with medicine, BrdU-positive cells increased obviously. At week 2, cell count of Huogu Ⅱ group plus Ach distinctively exceeded model group, while that data of Huogu Ⅱ group was not statistically higher than model group. The stem cell finished homing could possibly differentiate into osteocytes and vascular endothelial cells under the inducement of local environmental factors. In histopathologic examination, significantly increased repair could be observed. In addition, the empty lacuna rate of Huogu Ⅱ Formula plus Ach group decreased more markedly than that of Huogu Ⅱ group. It indicated that Ach could further promote stem cell homing and the initiation of homing was relatively earlier.

SDF-1 is the first reported chemoattractant protein that participates in the whole homing course of stem cells and progenitor cells. Furthermore, homing of stem cells is dependent on the SDF-1 concentration gradient.[18] In this study, we found that, on the 2nd week, the expression of SDF-1 was significant, especially in the ischemic necrosis tissue of femoral head. The expression exists in vascular endothelial cells, especially the cells of repaired region, as well as in bone marrow stromal cells. These findings indicate that up-regulation of SDF-1 expression in ischemic area medicates the course of BMSCs homing to ischemic tissue. The reason that the SDF-1 expression of week 4 was lower than week 2 was possibly because the differentiation of activated stem cells had been accomplished or the repair reaction of liquid nitrogen-induced ONFH started to decline. Huogu Ⅱ is able to up-regulate the positive expression of SDF-1 drastically, improve the SDF-1 concentration gradient between ischemic area and bone marrow, and thereby facilitate BMSCs homing activity. This effect was enhanced after addition with Ach and the difference was clearly observed in terms of SDF-1 level at week 2, which is consistent with BrdU-positive cell count increased significantly at the same time point. The information mentioned above suggests that Ach might promote BMSCs homing to necrosis lesion through increasing SDF-1 level in pathologic tissues and consequently boost the repair activity there.

Previous studies have proven that inducement by SDF-1 and other factors activated BMSCs homing to pathologic tissue.[19] At present, several aspects of the repair mechanism of BMSCs after homing to affected parts were understood. On one hand, they differentiate into tissue cells in situ under the inducement of the local microenvironment. For instance, they differentiate into osteocytes and vasculr endothelial grouth factors (VEGFs), which participate in the bone repair and vascular regeneration in the pathologic area. On the other hand, in the local focus, they secret cytokines that are of bone-repair-facilitating or vascular-regeneration-promoting effects, such as BMP2, VEGF, and SDF-1. BMP2 is a powerful osteogenic factor. SDF-1 regulates the migration and homing of BMSCs and also plays an important role in tissue repair after injury. To some extent, similar mechanisms were observed in this study through pathologic and molecular biological methods.

To sum up, by studying the influence of Huogu Ⅱ Formula plus Ach (a medicinal guide) on BMSCs homing to necrosis focus in ONFH rabbit induced by liquid nitrogen freezing, we primarily expound the mechanism of Ach in enhancing curative effects of ONFH by promoting BMSCs of peripheral blood homing to the necrosis lesion. Related researches suggest that its effect of facilitating BMSCs homing to pathologic area could possibly be the biological manifestations of Ach guiding other drugs to the affected part.

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(Received May 5, 2011)
Edited by YUAN Lin