

*Full Length Research Paper*

## Effect of ultra-short wave on hormone-induced ischemic necrosis of femoral head in early stage

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Received 17 January, 2014; Accepted 16 May, 2014

This study aims to investigate the effect of ultra-short wave on hormone-induced ischemic necrosis of femoral head in early stage by using an Avascular necrosis of the femoral head (ANFH) rabbit model. Horse serum was administered to male rabbits via the marginal ear vein, whereas control rabbits received saline. After five weeks, the rabbits with horse serum were injected with methylprednisolone. Blood-lipoids and hemodynamic parameters were analyzed. Collagen type I was analyzed by using immunohistochemistry. Pathological examination of femoral head was analyzed by hematoxylin-eosin (HE) staining; Expression of vascular endothelial growth factor (VEGF) and BMP-2 mRNA was analyzed by RT-PCR. Results revealed that when compared with control group, the difference in model group was significant ( $P < 0.05$  or  $P < 0.01$ ), with regard to TXB<sub>2</sub>, 6-keto-PGF<sub>1</sub> $\alpha$ , blood fat level, T/P, and hemodynamic parameters, while compared with model group, the difference of USW group was significant ( $P < 0.05$  or  $P < 0.01$ ). The expression of collagen type I was significantly lower in the model animals than that in the controls, while that of the USW diathermy group was significantly higher than the control group ( $P < 0.05$ ). Pathology index was significantly different in the USW diathermy animals than that in the controls ( $P < 0.05$ ). Real-time PCR showed that VEGF mRNA and BMP-2 mRNA levels in the model group were higher significantly as compared to those in the control group ( $P < 0.01$ ), while that in the diathermy group were significantly higher than those in the model group ( $P < 0.01$ ). The study thus revealed that ultra-short wave has a positive curative effect on ANFH.

**Key words:** Ultra-short wave, Avascular necrosis of the femoral head (ANFH), Collagen Type I, VEGF mRNA, BMP-2 mRNA.

### INTRODUCTION

Avascular necrosis of the femoral head (ANFH) is a pathologic process that results from interruption of blood supply to the bone which might lead to eventual collapse of bone structure if there is no immediate intervention

(Shi et al., 2010). Femoral head osteonecrosis is common in China (Zhao and Wang, 2007). The etiology of femoral head osteonecrosis involves trauma and non-traumatic factors such as corticosteroid use, high alcohol

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intake, genetic predisposition, Gaucher's disease, radiation, chronic pancreatitis, HIV as well as idiopathic causes (Miller et al., 2002). Hormone therapy is the most common cause of non-traumatic ANFH (Assouline-Dayana et al., 2002).

Hormone, especially corticosteroid, has been widely used in clinic. Although corticosteroid can provide striking effects on various diseases, it also could have adverse effects including growth retardation, obesity, osteoporosis, hyperglycemia, osteonecrosis, cataract, and Cushing syndrome, (Ahmed et al., 2012) particularly after high dosages and long-term use (Gulati et al., 2003; Hougardy et al., 2000). Recently, more cases of ANFH induced by corticosteroid have been reported. The therapeutic effect of ANFN is often not satisfactory, while patients with ANFH usually end up with hip arthroplasty. So it is worthwhile to prevent and cure ANFH before the lesion reaches the stage when arthroplasty becomes an inevitable option. In the early stage, there are several options, such as anti-lipid drugs and Chinese medicine, but the effect is often not satisfactory (Dotti et al., 2002).

The application of ultra-short wave (USW) therapy in rehabilitation medicine has become more and more extensive, especially in peripheral circulatory system (Zhang et al., 2008). USW therapy can resolve inflammatory states, reduce swelling, promote vasodilation, and ameliorate blood circulation (Pang et al., 2013). Thus, USW was widely used in acute or chronic inflammatory disease, and have a positive effect. However, the effect of ultra-short wave on ANFN femoral head injury was not completely understood. In this study, we used horse serum and hormone to establish ANFN rabbit model. Then, we evaluated the effects of USW on ANFN femoral head injury in early stage. In addition, the potential mechanism was also investigated.

## MATERIALS AND METHODS

Animal experiments were conducted in accordance to the Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised 1985) and the Regulations of the Committee on the Use and Care of Animals of Shandong University in Jinan, China. This study was approved by the Ethics Committee of the Second Hospital of Shandong University in Jinan, China.

### Chemicals and reagents

Methylprednisolone was purchased from US (Chinese Drug Approval Number: H20040338). Horse serum was purchased from US. Immunohistochemical kit of streptavidin biotin-peroxidase complex (SABC) and collagen Type I polyclonal antibody of rabbit were both purchased from Tianlai Biotechnology Co. Ltd (Beijing, PRC)

### Animals and experimental proceeding

Male New Zealand rabbits aging 24 weeks and weighing 2.5 to 3.0 kg were provided by the Laboratory Animal Center of Shandong

University (Jinan, Shandong, China). Prior to the experiments, animals were adapted to new environment for one week. In the present study, animals were housed in separate cages under diurnal lightning conditions with free access to food and water.

After acclimatization, the animals were randomly divided into 3 groups: blank control group (group A), model group (group B) and experiment group (group C) (n =10 in each group). Rabbits in model and experiment group were injected with horse serum (15 ml/kg) via the ear vein in the first and third week. Meanwhile, the control rabbits received saline. After five weeks, the rabbits receiving horse serum were injected with methylprednisolone (40 mg/kg) intraperitoneally daily for three days. Rabbits in control group received the same dose of saline. The rabbits in experiment group were treated with USW diathermy treatment from the sixth week. The USW therapy apparatus is floor-type LDT-CD31 electrotherapy machine (Shanghai, China). It has a frequency of 40.68 MHz, the wavelength of 7.3 m, the utmost output power of 200w and two electrodes of 20 cm multiplied by 15 cm. The electrodes are put both sides of the hip of a controlled rabbit with a gap of 2 to 3 cm between the electrode and the skin. Tiny quantity of heat is needed. Continuous treatment lasts 3 weeks, 15 min once per day, to an end in the 9th week.

### Observation of general condition

The weight changes, color and luster of hair and tail, and activity of all the rabbits were recorded.

### Blood-lipoids and hemodynamic parameters

In the 10th week, 3 ml venous blood was obtained from marginal ear vein of each rabbit. Then blood was centrifuged at 3000 rpm for 10 min to collect serum. Total cholesterol (TCH) and triglyceride (TG) were detected by the blood cell analyzer using partial serum, and the rest was reserved at -20°C. Serum thromboxane B2 (TXB2, the stable metabolites of TXA2) and 6-Keto prostaglandin F1 alpha (6-keto-PGF1α, the stable metabolites of PGI2) were detected with the EIA kit (Cayman co. Ltd). TXB2/6-keto-PGF1α (T/P) representing balance of TXA2-PGI2 was calculated. Venous blood (4 ml) was obtained from the opposite side of marginal ear vein, mixed with heparin to prevent blood coagulation. Hemodynamic parameters were detected using fully automatic blood rheometer (FASCO-3010A).

### Immunohistochemistry for collagen Type I

At the end of the 12<sup>th</sup> week, the rabbits were sacrificed through air embolism. Then bilateral femoral head of rabbit tissue was taken immediately. Femoral head tissues were stored in 10% formalin, fixed at room temperature for 36 ~ 72 h. The fixed tissue was decalcified with 20% EDTA (ethylene diamine tetraacetic acid) for preparation of paraffin section. The section was dewaxed and washed with phosphate buffer solution (PBS) flushing to repair tissue antigen. The first antibody, biotinylated sheep-anti-rabbit horseradish peroxidase (HRP) polymer and avidin-biotinylated-enzyme complex (ABC) were added, and colored using hydrogen peroxide after PBS flushing. The reaction was terminated until the orange-yellow granules occurred under microscope. The sections were stained with hematoxylin, dehydrated with alcohol, and sealed with neutral resins. We observed the immune complex deposition area and strength of immune response. The orange-yellow flake or granular staining was considered as standard positive reaction. The collagen Type I level was positively related to the intensity of immunohistochemical staining grading five levels.

**Table 1.** Oligonucleotide sequences of primers.

Gene	Sequence	
VEGF	Forward primer	5'-AATGATGAAAGCCTGGAGTGTG-3'
	Reverse primer	5'-TCACATTTGTTGTGCTGTAGGAAG-3'
BMP-2	Forward primer	5'-TTTGGTCAACTCCGTGAACTCTAA-3'
	Reverse primer	5'-ACACCCACAACCCTCCACA-3'
$\beta$ -actin	Forward primer	5'-TGCGGGACATCAAGGAGAA-3'
	Reverse primer	5'-AGGAAGGAGGGCTGGAACA-3'

### Pathological examination of femoral head

The fixed tissue was decalcified in 5% salpeter solution for one week, dehydrated using graded ethanol, wrap with wax immersion, and embedded in paraffin. Then it was sliced into sections up to about 5  $\mu$ m thick using a microtome, and the sections were dewaxed, stained by routine HE staining. Morphological changes of osteoblast and osteoclast, the empty cartilage cell lacunae, and the density of blood vessels under the cartilage were observed. Meanwhile, the thickness of femoral head cartilage, the width of bone trabeculae, the diameters of fat cells and the adipocyte area rates were analyzed using image analysis system.

### Real-time PCR analysis of the femoral head tissues

Femoral head was frozen quickly through liquid nitrogen and reserved in cryogenic refrigerator. The expression of VEGF and BMP-2 mRNA in the femoral head was detected by quantitative real-time PCR. The oligonucleotide sequences of the primers were synthesized by TaKaRa Bio (China) (Table. 1). And all the reagent kits used bellow were provided by TaKaRa Bio (China). Total RNA was extracted with Trizol reagent (Invitrogen, USA). Then the purified RNA was diluted up to 500 ng  $\mu$ l<sup>-1</sup> and 3  $\mu$ l RNA was utilized to synthesize cDNA with Prime-Script RT reagent kit. Following manufacturer's instruction, the mixture was incubated at 37°C for 45 min, 85°C for 5 s and 4°C for 7 min. Real-time PCR was performed with SYBR1Premix Ex Taq TM kit and qPCR system. The cycling parameters were set as follows: initial denaturation at 95°C for 30 s, followed by 40 cycles at 95°C for 5 s and 60°C for 30 s, and final melting curve analysis for distinguishing main PCR products from primer dimers. The cycle number where the amplification curve crossed the threshold line was noted as the critical threshold (CT) and the gene expression was calculated by the comparative C<sub>T</sub> ( $2^{-\Delta\Delta C_T}$ ) method, using  $\beta$ -actin as the control housekeeping gene for normalization.

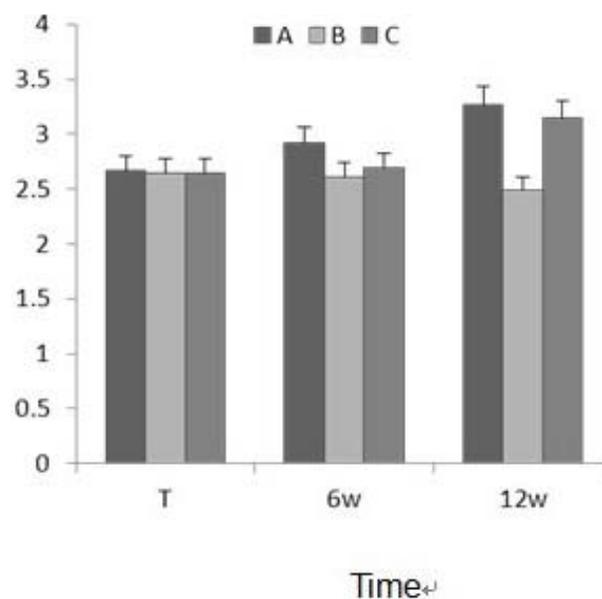
### Statistical analysis

All data were presented as means  $\pm$  SD and analyzed with one-way analysis of variance (ANOVA) and Student's t-test. P value < 0.05 was considered statistically significant.

## RESULTS

### General condition

After induction of ANFN, all ANFN groups showed less



**Figure 1.** The ultra-short wave improved the weight following ANFN. The weight was measured as described in "Materials and Methods". The aggregate score represents the average weight per animal (n=20 for each group). \*P < 0.01 vs. Group A,  $\Delta$ P < 0.05 vs. Group B. Group A: control; Group B: vehicle treated ANFN; Group C: ultra-short wave treated ANFN.

weight growth as compared with control group. After USW therapy, weight of rabbits grew more as compared with the model group (Figure 1). Rabbits of model group were depressed, and had fluffy lackluster fur, awkward hind limbs, short bounce distance and height. In contrast, the rabbits after treatment, which had glossy fur, sensitive activity, and long bounce distance and height, were active. The USW therapy significantly improved the weight and motility.

### Effect of USW on blood-lipids and hemodynamic parameters

When compared with control group, the difference of

**Table 2.** TXB2, 6-keto-PGF1 $\alpha$ , T/P, blood fat level analysis (mean $\pm$ SD).

Group	TXB2(pg/ml)	6-keto-PGF1 $\alpha$ (pg/ml)	T/P	TG(mmol/L)	TCH(mmol/L)
A	170.5 $\pm$ 39.8	241.6 $\pm$ 52.3	0.6 $\pm$ 0.2	0.7 $\pm$ 0.2	1.5 $\pm$ 0.5
B	450.1 $\pm$ 108.6*	140.3 $\pm$ 25.2*	3.2 $\pm$ 0.5*	3.0 $\pm$ 0.8*	4.1 $\pm$ 0.4*
C	227.1 $\pm$ 52.5* $\Delta$	235.6 $\pm$ 71.8* $\Delta$	0.9 $\pm$ 0.2* $\Delta$	1.3 $\pm$ 0.4* $\Delta$	2.6 $\pm$ 0.6* $\Delta$

\*P < 0.05 vs. Group A;  $\Delta$ P < 0.05 vs. Group B. Group A: control; Group B: vehicle treated ANFN; Group C: ultra-short wave treated ANFN.

**Table 3.** Hemodynamics analysis (mean $\pm$ SD).

Group	WBLSV (mPa.s)	WBHSV (mPa.s)	PV (mPa.s)	Equation K value	EDI
A	6.33 $\pm$ 0.58	4.21 $\pm$ 0.32	1.56 $\pm$ 0.21	13.91 $\pm$ 4.18	0.79 $\pm$ 0.09
B	11.27 $\pm$ 0.75*	6.98 $\pm$ 0.50*	2.02 $\pm$ 0.46*	38.16 $\pm$ 1.79*	0.90 $\pm$ 0.03
C	8.70 $\pm$ 0.71* $\Delta$	5.03 $\pm$ 0.38* $\Delta$	1.70 $\pm$ 0.32	30.12 $\pm$ 2.01* $\Delta$	0.93 $\pm$ 0.11

\*P < 0.05 vs. Group A;  $\Delta$ P < 0.05 vs. Group B. Group A: control; Group B: vehicle treated ANFN; Group C: ultra-short wave treated ANFN. WBLSV: whole blood low sheathed viscosity; WBHSV: whole blood high sheathed viscosity; PV: plasma viscosity; EDI: erythrocyte deformability index

**Table 4.** Immunohistochemistry staining analysis (number).

Group	Number	immunohistochemistry staining intensity				
		++++	+++	++	+	-
A	20	0	3	17	0	0
B	20	0	0	2	18	0
C	20	2	15	3	0	0
Total	60	2	18	22	18	0

Comparison between groups was statistically significant (P<0.05).++++: super strong positive reaction; +++: strong positive reaction; ++: moderate positive reaction; +: weakly positive reaction; -: negative reaction.

model group was significant (P<0.05 or P<0.01), with regard to TXB2, 6-keto-PGF1 $\alpha$ , blood fat level, T/P, and hemodynamic parameters. Compared with model group, the difference of USW group was significant (P<0.05 or P<0.01). They improved the 6-keto-PGF1 $\alpha$  of cases, and decreased TXB2, blood fat level, T/P, (Table 2) and hemodynamic parameters (Table 3) respectively. The animals in the model group were found with ANFN manifesting avascular necrosis of femoral head bone cells, decrease of bone marrow cells, high blood viscosity and imbalance of TXA2-PGI2. The USW diathermy group demonstrated significant improvement of blood supply, TG level and blood viscosity, as well as PLT activity and the TXA2-PGI2 ratio.

#### Effects of USW therapy on collagen Type I

The expression of collagen Type I was significantly lower in the model animals than that in the controls as indicated

by the stronger immunohistochemistry staining for rabbits collagen type, while that of the USW diathermy group was significant higher than the control group (P<0.05) (Table 4) USW diathermy can promote the expression of collagen Type I.

#### Effect of USW therapy on pathological changes of femoral head

The amount of osteoblast in the model group was significantly lower than control group, while in the diathermy group it was significantly increased compared with the model group (P<0.01). The amount of osteoclast in the model and diathermy group was significantly higher than in the control group, and in the diathermy group it was significantly higher than in the model group (P<0.05). The empty cartilage cell lacunae ratios of the model and diathermy groups were significantly higher than in the control group, and the diathermy group showed significant

**Table 5.** Pathology of femoral head indexes analysis (mean±SD).

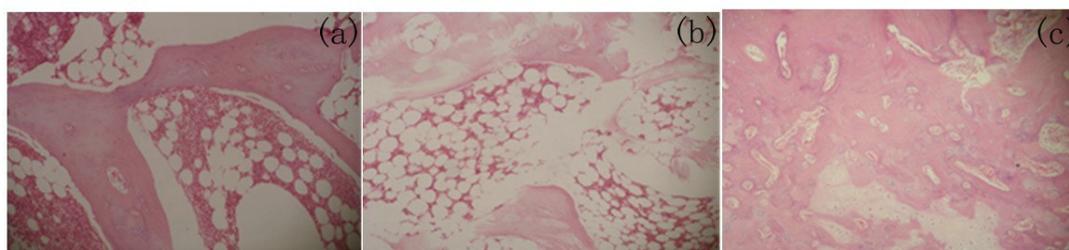
Group	Osteoblast (number)	Osteoclast (number)	The empty cartilage cell lacunae ratios (%)	Density of blood vessels under the cartilage (number)
A	21.00±3.63	4.75±1.59	6.51±1.47	11.55±2.16
B	14.75±3.68*	13.06±2.79*	20.29±2.64*	6.69±1.62*
C	36.00±6.02*Δ	8.61±2.45*Δ	9.18±1.85*Δ	14.06±2.15*Δ

\*P < 0.05 vs. Group A; ΔP < 0.05 vs. Group B.

**Table 6.** Pathology of femoral head indexes analysis (mean±SD).

Group	Thickness of femoral head cartilage (μm)	Width of bone trabeculae (μm)	Diameters of fat cells (μm)	Adipocyte area rates (%)
A	518.46±62.31	127.55±13.51	43.85±6.24	12.54±3.52
B	229.44±45.91*	73.06±16.61	63.44±7.33*	25.79±4.61*
C	334.50±49.22*Δ	102.89±10.17*	52.11±5.80*Δ	20.91±2.51*

\*P < 0.05 vs. Group A; ΔP < 0.05 vs. Group B.



**Figure 2.** Pathological changes of femoral head were observed. (a):group A: amount of hematopoietic cell was observed in medullary canal, and the ratio of fat cells was normal; (b):group B: bone trabecular was sparse, and the number and size of fat cells was increased;(c):group C: Osteoblasts were observed in bone trabecular, and new vessels were increased.

degradation compared to the model group ( $P < 0.05$ ). The density of blood vessels under the cartilage in the model group was significantly less compared with the control group, while in the diathermy group it was significantly increased compared with the model group ( $P < 0.01$ ) (Table 5).

The thickness of femoral head cartilage in the model and diathermy group was reduced compared with the control group, while it was thicker in the model group than in the diathermy group ( $P < 0.05$ ). The width of bone trabeculae in the model and diathermy group was significantly less compared with the control group, while they were significantly wider in the diathermy compared with the model group ( $P < 0.01$ ). The diameters of fat cells in the model and diathermy group were increased compared with the control group, while they were significantly smaller in the diathermy group compared with the model group ( $P < 0.05$ ). The adipocyte area rates in the model and diathermy group were significantly

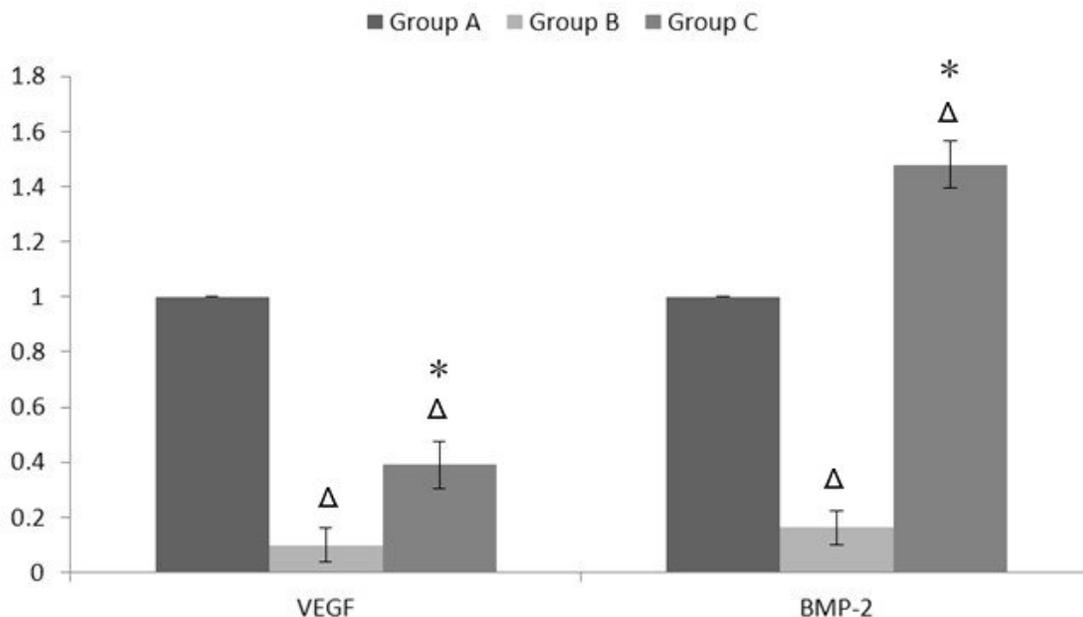
elevated compared with the control group, and rates in the model group were significantly elevated compared with the model group ( $P < 0.05$ ) (Table 6) (Figure 2).

### The effects of USW on the expression of VEGF and BMP-2 mRNA

Real-time PCR showed that in femoral head VEGF mRNA and BMP-2 mRNA levels in the model group were higher significantly as compared to those in the control group ( $P < 0.01$ ). Meanwhile, VEGF mRNA and BMP-2 mRNA levels in the diathermy group were significantly higher than those in the model group ( $P < 0.01$ ) (Figure 3).

## DISCUSSION

Hormone-induced ANFN will occur when femoral head



**Figure 3.** The ultra-short wave induced the expression of VEGF mRNA and BMP-2 mRNA. The weight was measured as described in "Materials and Methods". The mRNA expression was calculated by the  $2^{-\Delta\Delta CT}$  method using  $\beta$ -actin as the reference gene (mean $\pm$ SD). The relative mRNA levels were represented as the ratios by comparing the expression of each group (n=20 for each group). \*P < 0.01 vs. Group A,  $\Delta$ P < 0.01 vs. Group B. Group A: control; Group B: vehicle treated ANFN; Group C: ultra-short wave treated ANFN.

component (osteocyte, bone marrow hematopoietic cells, and fat cells) is dead caused by large dose hormone application. In this study, we have taken the advantage of an ANFN animal model to investigate the effects of USW on hormone-induced femoral head injury. It has been reported that horse serum could induce fat metabolism disorder and mild small arteritis in bone (Wang et al., 2011, Andersen et al., 2013). The hormone could aggravate the potential vasculitis (Zhang et al., 2013). The vasculitis of animal was caused by horse serum, and then a large dose of hormone was used to induce the typical model of osteonecrosis (Song et al., 2010). In the present study, we successfully induced ANFN in rabbits by intravenous injection of horse serum and methylprednisolone as confirmed by the appearance of osteonecrosis in the femoral head. Loss of weight, poor mental state and declination of exercise capacity were apparent after ANFN. USW improved the weight, mental state and exercise capacity.

In this study, sensitization of rabbits was caused by injection of horse serum, after that, the models were made by the use of hormone. Then we found that the blood-lipoids level was higher after the use of hormone suggesting that hormone could cause hyperlipidemia along with previous studies (Vuksanovic et al., 2006; Grover et al., 2007; Jones, 1992). TXA2 and PGI2 were metabolites of arachidonic acid in plaque lipid membrane. TXA2 could promote platelet aggregation, while PGI2 could inhibit platelet aggregation and diastole blood

vessels. The balance of TXA2 and PGI2 was important in regulation of platelet function and vascular tone (Wei et al., 1998). In this study, we found that hormone could increase platelet activity and TXA2 synthesis, decrease the PGI2 synthesis, and increase TXA2/PGI2 ratio. That would prompt thrombosis leading to osteonecrosis of the femoral head. USW effectively inhibited aggregation and activation of platelet in ischemia of tissue, and regulated the balance of TXA2/PGI2; however, but the underlying mechanism still requires further study. Through USW therapy, the hemodynamic parameters were improved, which indicated that ultra-short wave may improve microcirculation, promote the blood flow, and prevent the thrombosis.

The expression of collagen Type I mRNA, a specific molecular marker of early differentiation of osteoblast, was related with osteogenetic activity (Turner and Spelsberg, 1991). Glucocorticoids could inhibit the synthesis of collagen type I in a time- and concentration-dependent way (Delany et al., 1994). In this study, the immunohistochemistry staining of the collagen Type I was significantly lower in the model animals than that in the controls. We speculated that synthesis ability of collagen Type I in controls was decreased, to cause insufficient repair after osteonecrosis. The immunohistochemistry staining of the collagen Type I was significantly higher in the diathermy group than that in the controls. USW could alleviate or reverse the inhibit effect of methylprednisolone against collagen Type I to

maintain a good function of osteoblast.

It has been reported that USW could improve the blood flow (Mlynarik et al., 2008), and the improvement of blood flow was the ultimate aim of the treatment of femoral head necrosis in the early stage. In this study, the density of blood vessels under the cartilage in the diathermy group was significantly increased compared with the model group. We considered that the improvement of blood flow increased the cartilage thickness, promoted bone marrow stromal cells differentiate into preosteoblast and osteoblast, increased the source of osteoblast, and then broaden bone trabecular, reduced the osteonecrosis, effectively improved necrotic bone repairing, and prevented the femoral head necrosis. Otherwise, USW could also prevent the bone marrow stromal cells differentiate into fat cell, indirect inhibit intramedullary fat accumulation, reduce the pressure in the femoral head, improve the blood flow of femoral head, and prevent bone ischemia. In this study, we demonstrated that the thickness of femoral head cartilage, the width of bone trabeculae, the diameters of fat cells in the diathermy group than that in the controls.

It has been reported that VEGF and BMP-2 were the two most important factors affecting the repair of femoral head (Vadasz et al., 2004). Glucocorticoids may inhibit the expression of VEGF mRNA, which may participate in the pathological process of bone metabolic abnormalities (Pufe et al., 2003). In this study, VEGF mRNA and BMP-2 mRNA levels in the model group were elevated significantly as compared to those in the control group. That indicated that hormone was strongly inhibit the VEGF synthesis, as it was reported (Li et al., 2005; Gloddek et al., 1999). VEGF mRNA and BMP-2 mRNA levels in the diathermy group were significantly higher than those in the model group. That proved that the USW could induce the expression of VEGF mRNA and BMP-2 mRNA, and enhanced the repair of femoral head after ANFN.

## Conclusion

In our study, these results showed that USW had a positive curative effect on ANFH by promoting biochemical metabolism of bone cells, promoting osteoblastic proliferation and differentiation, improving the blood flow, enhancing the repair of femoral head and so on. USW may be a useful remedy for the treatment of ANFH in the early stage. However, in this study, we used microcalorimetry ultra-short wave therapy, and the function of different dosage USW was unclear, so further studies should be performed to provide more experimental data to support its clinical application.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

## REFERENCES

- Ahmed HH, Morcos NY, Eskander EF, Seoudi DM, Shalby AB (2012). Potential role of leptin against glucocorticoid-induced secondary osteoporosis in adult female rats. *Eur. Rev. Med. Pharmacol. Sci.* 16:1446-52. PMID:23104664
- Andersen DC, Nielsen C, Jensen CH, Sheikh SP (2013). Horse serum reduces expression of membrane-bound and soluble isoforms of the preadipocyte marker Delta-like 1 homolog (Dlk1), but is inefficient for adipogenic differentiation of mouse preadipocytes. *Acta Histochem.* 115:401-6. <http://dx.doi.org/10.1016/j.acthis.2012.08.005>; PMID:22975115
- Assouline-Dayana Y, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME (2002). Pathogenesis and natural history of osteonecrosis. *Semin. Arthritis Rheum.* 32:94-124. <http://dx.doi.org/10.1053/sarh.2002.33724b>; PMID:12430099
- Delany AM, Dong Y, Canalis E (1994). Mechanisms of glucocorticoid action in bone cells. *J. Cell. Biochem.* 56:295-302. <http://dx.doi.org/10.1002/jcb.240560304>; PMID:7876321
- Dotti R, Muller DM, Benini A (2002). Clinical aspects, etiology, pathogenesis, diagnosis and therapy of aseptic bone necrosis - A current analysis of the literature. *Praxis (Bern 1994)*, 91:163-76.
- Gloddek J, Pagotto U, Paez PM, Arzt E, Stalla GK, Renner U (1999). Pituitary adenylate cyclase-activating polypeptide, interleukin-6 and glucocorticoids regulate the release of vascular endothelial growth factor in pituitary folliculostellate cells. *J. Endocrinol.* 160:483-90. <http://dx.doi.org/10.1677/joe.0.1600483>; PMID:10076194
- Grover GJ, Mellstrom K, Malm J (2007). Therapeutic potential for thyroid hormone receptor-beta selective agonists for treating obesity, hyperlipidemia and diabetes. *Curr. Vasc. Pharmacol.* 5:141-54. <http://dx.doi.org/10.2174/157016107780368271>; PMID:17430219
- Gulati S, Godbole M, Singh U, Gulati K, Srivastava A (2003). Are children with idiopathic nephrotic syndrome at risk for metabolic bone disease? *Am. J. Kidney Dis.* 41:1163-9. [http://dx.doi.org/10.1016/S0272-6386\(03\)00348-2](http://dx.doi.org/10.1016/S0272-6386(03)00348-2)
- Hougardy DM, Peterson GM, Bleasel MD, Randall CT (2000). Is enough attention being given to the adverse effects of corticosteroid therapy? *J. Clin. Pharm. Ther.* 25:227-34. <http://dx.doi.org/10.1046/j.1365-2710.2000.00284.x>; PMID:10886467
- Jones JJ (1992). Intravascular coagulation and osteonecrosis. *Clin. Orthop. Relat. Res.* 41:53. PMID:1532547
- Li X, Jin L, Cui Q, Wang GJ, Balian G (2005). Steroid effects on osteogenesis through mesenchymal cell gene expression. *Osteoporos. Int.* 16:101-8. <http://dx.doi.org/10.1007/s00198-004-1649-7>; PMID:15205891
- Miller KD1, Masur H, Jones EC, Joe GO, Rick ME, Kelly GG, Mican JM, Liu S, Gerber LH, Blackwelder WC, Falloon J, Davey RT, Polis MA, Walker RE, Lane HC, Kovacs JA (2002). High prevalence of osteonecrosis of the femoral head in HIV-infected adults. *Ann. Int. Med.* 137:17-25. <http://dx.doi.org/10.7326/0003-4819-137-1-200207020-00008>; PMID:12093241
- Mlynarik V, Kohler I, Gambarota G, Vaslin A, Clarke PG, Gruetter R (2008). Quantitative proton spectroscopic imaging of the neurochemical profile in rat brain with microliter resolution at ultrashort echo times. *Magn. Reson. Med.* 59:52-58. <http://dx.doi.org/10.1002/mrm.21447>; PMID:18050343 PMID:PMC2391255
- Pang CJ, Tong L, Ji LL, Wang ZY, Zhang X, Gao H, Jia H, Zhang LX, Tong XJ (2013). Synergistic effects of ultrashort wave and bone marrow stromal cells on nerve regeneration with acellular nerve allografts. *Synapse* 67(10):637-647. <http://dx.doi.org/10.1002/syn.21669>; PMID:23554017
- Pufe T, Scholz-Ahrens KE, Franke AT, Petersen W, Mentlein R, Varoga D, Tillmann B, Schrezenmeier J, Güter CC (2003). The role of vascular endothelial growth factor in glucocorticoid-induced bone loss: evaluation in a minipig model. *Bone* 33:869-876. <http://dx.doi.org/10.1016/j.bone.2003.08.002>; PMID:14678846
- Shia B, Li G, Wang P, Yin W, Sun G, Wu Q, Yu G (2010). Effect of antler extract on corticosteroid-induced avascular necrosis of the femoral head in rats. *J. Ethnopharmacol.* 127:124-129. <http://dx.doi.org/10.1016/j.jep.2009.09.036>; PMID:19818844

- Song HJ1, Lan BS, Cheng B, Zhang KF, Yan HW, Wang WZ, Gao ZQ (2010). Peripheral blood stem cell transplantation for ischemic femoral head necrosis. *Transplant Proc.* 42:1862-1864.
- Turner RT, Spelsberg TC (1991). Correlation between mRNA levels for bone cell proteins and bone formation in long bones of maturing rats. *Am. J. Physiol.* 261:E348-353. PMID:1887882
- Vadasz Z, Misselevich I, Norman D, Peled E, Boss JH (2004). Localization of vascular endothelial growth factor during the early reparative phase of the rats' vessels deprivation-induced osteonecrosis of the femoral heads. *Exp. Mol. Pathol.* 77:145-8. <http://dx.doi.org/10.1016/j.yexmp.2004.06.002>; PMID:15351238
- Vuksanovic M, Beljic T, Popovic S (2006). Influence of hormone replacement therapy in postmenopausal women with type 2 diabetes and hyperlipidemia on lipid and glucose metabolism. *Srp Arh Celok Lek.* 134:295-301. <http://dx.doi.org/10.2298/SARH0608295V>; PMID:17009607
- Wang X, Reece SP, Van Scott MR, Brown JM (2011). A circadian clock in murine bone marrow-derived mast cells modulates IgE-dependent activation in vitro. *Brain Behav. Immun.* 25:127-34. <http://dx.doi.org/10.1016/j.bbi.2010.09.007>; PMID:20854894
- Wei XR, Hu L, Du J (1998) Effect of captopril on platelet cytosolic [Ca<sup>2+</sup>]<sub>i</sub> and plasma TXA<sub>2</sub>/PGI<sub>2</sub> in renovascular hypertensive rats. *Zhongguo Yao Li Xue Bao*, 19:89-91. PMID:10375769
- Zhang LX, Tong XJ, Sun XH, Tong L, Gao J, Jia H, Li ZH. (2008). Experimental study of low dose ultrashort wave promoting nerve regeneration after acellular nerve allografts repairing the sciatic nerve gap of rats. *Cell. Mol. Neurobiol.* 28:501-509. <http://dx.doi.org/10.1007/s10571-007-9226-1>; PMID:18058221
- Zhang Y, Wang X, Yang Z, Zhang H, Liu M, Qiu Y, Guo X (2013). The therapeutic effect of negative pressure in treating femoral head necrosis in rabbits. *PLoS One* 8(1):e55745. <http://dx.doi.org/10.1371/journal.pone.0055745>; PMID:23383276 PMCid:PMC3561314
- Zhao DW, Wang BJ (2007). Treatment of osteonecrosis of femoral head with minimally invasive vascularized bone grafting. *Zhonghua Yi Xue Za Zhi* 87:2036-40. PMID:17925174