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Effects of ultrasound on vascular endothelial growth factor in cartilage, synovial fluid, and synovium in rabbit knee osteoarthritis

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Ultrasound is commonly used to treat knee osteoarthritis (KOA), which has unique advantages with regard to relieving pain and inflammation as well as delaying cartilage degeneration, but the underlying mechanisms are less clear. The study aimed to investigate the therapeutic effects of ultrasound on vascular endothelial growth factor (VEGF) expression in cartilage, the synovium, and synovial fluid (SF) in a rabbit model of KOA. Twenty-four New Zealand rabbits were randomly divided into ultrasound (group A), sham ultrasound (group B) and no-ACLT control groups (group C). Six weeks after undergoing anterior cruciate ligament transection (ACLT), group A was treated with ultrasound and group B was treated with sham ultrasound. Two weeks thereafter, the morphology of the synovium and cartilage were observed. Cartilage and synovium were scored using the Mankin scale and Krenn V scores, respectively. VEGF expression in the cartilage, SF, and synovium of ACLT knee joints was analyzed via immunohistochemistry, western blotting, and RT-PCR. Cartilage degeneration and synovitis were the most severe in group B and the least severe in group C. Similarly, Mankin scores and Krenn V scores were highest in group B and lowest in group C ($p<0.05$). There were also significant differences in the VEGF IOD of cartilage or synovium, VEGF protein content in SF, and VEGF mRNA expression in cartilage or SF ($p<0.05$). Ultrasound can relieve synovitis and delay cartilage degradation, and the mechanisms of ultrasound for the treatment of KOA may involve inhibition of the expression of VEGF in the synovium, SF, and cartilage.

Key words: Osteoarthritis; ultrasound; vascular endothelial growth factor; cartilage; synovial fluid (SF); synovium

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Abbreviations: ACLT, anterior cruciate ligament transection; EBM, evidence-based medicine; Knee OA, KOA; MMPs, matrix metalloproteinases; OA, osteoarthritis; SF, synovial fluid; TBST, Tween-20 buffer; TIMPs, tissue inhibitors of metalloproteinases; VEGF, vascular endothelial growth factor

INTRODUCTION

In the human body, the site that exhibits the highest osteoarthritis (OA) morbidity is the knee. Knee OA (KOA) is reportedly present in 10% of the population aged over 55 years in Europe and America (Peat *et al.*, 2001), and one quarter of these individuals are severely disabled. In China, approximately 50% of KOA patients reportedly exhibit some degree of disability (Tan *et al.*, 2012). Pain is the primary reason for KOA patients consulting doctors, and the pathological changes involved in OA include synovitis, plica hyperplasia, and various degrees of cartilage damage (Wieland *et al.*, 2005). Thus, in patients in which surgery is unwarranted or contraindicated, the primary aims of treatment are pain relief, reducing synovitis, and delaying cartilage degradation.

Non-surgical KOA treatment methods include drugs and physical therapies designed to relieve pain and address pathological changes in the synovium and cartilage. Some existing methods are associated with side effects, for example the administration of nonsteroidal anti-inflammatory drugs can induce upper gastrointestinal ulceration and even upper gastrointestinal hemorrhage (Lanas *et al.*, 2006), and they cannot prevent the progression of KOA. Repetitive injections of sodium hyaluronate to relieve pain can be time consuming, and some patients are prone to developing drug dependence (Kolarz *et al.*, 2003; Harris *et al.*, 2011; Rovati *et al.*, 2006). The results of the administration of autologous blood derivatives are inconsistent, and there is a lack of evidence-based medicine (EBM) in this regard (Andia & Maffulli, 2013). The administration of herbs prescribed by practitioners of traditional Chinese medicine to treat KOA can also be time consuming, and can result in injury to the gastrointestinal tract. Externally applied herbs have inefficient absorption rates, resulting in low amounts of drugs being delivered to the articular cavity.

Since pain is the primary reason for KOA patients to consult doctors, it suggests that pain relief is their primary aim. Aside from drugs, acupuncture and moxibustion played an essential role in the treatment of KOA-derived pain before the development of modern rehabilitation medicine, especially in China, but EBM studies have found that acupuncture for KOA has relatively short-term clinical effects, and even these may be placebo effects (Manheimer *et al.*, 2007). It has been reported that in some studies moxibustion was effective and safe for the prevention and treatment of KOA (Beckwée *et al.*, 2015; Gaught & Carneiro, 2013), but moxibustion is a procedurally complex and time consuming treatment

(20–30 min per session), and there is a relatively high risk of burn injury (especially in the patients with combined nerve injury around the knee) compared to other thermal therapies.

Many basic and clinical KOA studies of modern rehabilitation interventions have reported that various modalities can reduce pain to some degree, but most of them do not constitute EBM. With regard to pathological changes in OA, superficial thermotherapy (hot packs or infrared therapy) cannot act on cartilage or subchondrally. Ultrashort waves can affect deep tissues, which may result in radiation-associated side effects. The main mode of action of magnet therapy is the facilitation of bone metabolism, and thus it has little therapeutic effect on cartilage and synovium, and it also takes a long time to affect tissues. The main mechanism of transcutaneous electrical nerve stimulation is stimulation of peripheral nerve fibers and inhibition of afferent pain, but it cannot delay the progression of OA. Functional electrical stimulation/neuromuscular electrical stimulation or medium frequency electrotherapy can induce passive contraction of muscles of the knee joint and improve blood circulation, but the contraction model cannot enhance joint stability, and with regard to circulation cartilage does not contain blood vessels and there is minimal blood flow in the synovium; thus the modality cannot delay cartilage degeneration.

Much research has reported that ultrasound can relieve pain, increase range of motion, and improve joint function, especially in patients with mild or moderate KOA (Loyola-Sánchez *et al*., 2010; Yeğin *et al*., 2017; Bashardoust Tajali *et al*., 2012). Ultrasound has deep heat effects, mechanical vibration effects, and cavitation effects that relieve adhesion of tissues and affect the metabolism of cartilage. Compared with other modalities, it is superior for pain relief and delaying pathological changes in KOA patients. It has also been reported that ultrasound was superior to millimeter waves, pulsed electromagnetic fields, ultrashort waves, and low-power lasers for reducing chondrocyte apoptosis and regulating the expression of apoptosis genes (Guo *et al*., 2011), and the session duration of ultrasound therapy (5–10 min) is shorter than that of other therapies (20–40 min).

Though ultrasound is commonly used to treat KOA in China and other countries, and some studies have investigated the mechanisms of ultrasound treatment in KOA, most of these studies have focused on the effects of ultrasound on inflammatory factors and molecular and cellular components related to cartilage metabolism such as matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs) (Yeğin *et al*., 2017), and chondrocyte apoptosis (Zeng *et al*., 2012). Synovitis also plays an important role in the development of OA, but few studies have investigated the effects of ultrasound on the synovium and synovial fluid (SF), and there are reports on the effects of ultrasound on vascular endothelial growth factor (VEGF) in cartilage, the synovium, and SF. Some studies suggest that VEGF can enhance the secretion of MMPs, and induce cartilage degeneration by inhibiting the synthesis and expression of proteoglycan and type II collagen (Ren *et al*., 2012). It has also been reported that VEGF is involved in specific pathological changes associated with joint pain, including cartilage degeneration, synovitis, and the formation of osteophytes and subchondral bone cysts (Zhang *et al*., 2016). Reducing the amount of VEGF in cartilage, the synovium, and SF may relieve pain in KOA patients.

In the present study conducted using a rabbit model of KOA, the morphology of synovium and cartilage was observed via the naked eye and light microscopy, cartilage was scored using the Mankin scale, and synovium was assessed *via* Krenn V scores. VEGF expression levels in the cartilage, SF, and synovium of ALCT knee joints were assessed after ultrasound treatment using immunohistochemistry, western blotting, and RT-PCR to investigate the potentially therapeutic effects of ultrasound on KOA, and the mechanisms involved.

MATERIALS AND METHODS

Antibodies and chemical reagents. Rabbit anti-TIMP-1 and anti-VEGF polyclonal antibodies were obtained from Beijing Biosynthesis Biotechnology (Beijing, China). Total RNA extraction reagents and SYBR Green qPCR Master Mix were purchased from Vazyme Biotechnology (Nanjing, China). RIPA lysis buffer, a BCA protein assay kit, an ECL chemiluminescence kit, and a hematoxylin and eosin staining kit were supplied by Beyotime Biotechnology (Shanghai, China). The DouSPTM IHC kit, DAB chromogenic kit, and neutral balsam were obtained from Maxim Biotechnology (Shanghai, China).

Rabbit KOA model. All animal procedures were performed in accordance with the relevant laws and institutional guidelines (animal license number No.SCXK (Hu) 2016-0011). Twenty-four healthy adult New Zealand white rabbits (age 6 months, mean weight 3.0 kg) were used. After 1 week of acclimation, the rabbits were randomly assigned to an ultrasound group (group A, n=8), a sham ultrasound group (group B, n=8), or a control group (group C, n=8). Groups A and B underwent anterior cruciate ligament transection (ACLT) to induce the KOA model, as previously described (Levillain *et al*., 2015; Schneider *et al*., 2015). Briefly, the rabbits in groups A and B were anesthetized with 5% chloral hydrate (5 mL/kg intraperitoneally) and treated aseptically throughout the experiment. After careful shaving and disinfection, ACLT was performed on the right knee of the hind leg via a lateral approach, and the left knee was left intact. Rupture of the anterior cruciate ligament was evaluated using the anterior drawer sign before closure of the articular capsule. The operated leg was not immobilized, and rabbits were permitted to move freely in their individual cages. Each day for 3 days after the surgery, 15 mg/kg of gentamycin was injected intramuscularly into the buttock. For 2 weeks after the surgery, the rabbits were monitored daily with regard to diet, mental status, stools, and urine. All animal experimental procedures were approved by the Ethics Committee of The Fifth Affliated Hospital of Guangzhou Medicine University.

Ultrasound therapy. Six weeks after the surgery, the rabbits in groups A and B were anesthetized via intraperitoneal injection and immobilized on an operating table. Hair around the right knee was removed to expose the right knee joint, and flexion of the knee was performed. The rabbits in group A were administered ultrasound therapy at a frequency of 3 MHz, intensity of 400 mW/cm^2, and pulse on-off ratio of 40%. The ultrasonic probe was moved along the line of the inner and outer knees at a speed of 1–2 cm/s for 10 min. One course of treatment consisted one session per day for 6 consecutive days. Each rabbit in group A received two courses of treatment. The rabbits in group B underwent the same procedure as those in group A, except the ultrasonic intensity was set to 0. The rabbits in group C did not receive any treatment.

Table 1. Scheme for the histopathological assessment of chronic synovitis

	Density of the resident cells	Inflammatory infiltration	Enlargement of the synovial lining cell layer
	0 points	0 points	0 points
	1 point	1 point	1 point
	2 points	2 points	2 points
Summary score	3 points	3 points	3 points

Summary score key: 0–1, no synovitis; 2–4, low-grade synovitis; 5–9, high-grade synovitis

Tissue sample collection. The rabbits were killed by air injection into the auricular vein. After careful dissection of the knees, SF, the synovium, cartilage, and condyle cartilage were harvested and stored under appropriate conditions prior to subsequent experiments.

Hematoxylin and eosin staining. The samples were dewaxed in xylene for 20 min and then rehydrated with an ethanol solution gradient (100%, 95%, 90%, 80%, 70%, and 0). The slides were stained with hematoxylin for approximately 2.5 min, rinsed in tap water, then incubated in hydrochloric acid alcohol for 1–3 seconds at room temperature. The slides were blued using bluing reagent for 30–60 seconds, rinsed in tap water, then incubated in eosin for approximately 1 min. Last, the slides were dehydrated with graded ethanol and xylene, and sealed with neutral balsam. Histology images were obtained using an optical microscope. Cartilage damage was evaluated using the modified Mankin method (Mankin *et al*., 1971), and the synovium was evaluated using the Krenn V method (Krenn *et al*., 2006) (see Table 1).

Immunohistochemical staining. Immunohistochemical staining was performed as described previously (Wagenaar-Miller *et al*., 2007; Yi *et al*., 2017). Antigen retrieval was performed using the proteinase K method. Endogenous peroxidase activity was quenched with 1% H_2O_2 for 15 min, followed by washing with tap water. Slides were incubated overnight at 4°C with the corresponding antibodies. The next day the slides were washed twice with 1× Tris-buffered saline with Tween-20 buffer (TBST). Peroxidase conjugated anti-mouse/anti-rabbit antibody was added to the slides and they were incubated for 1 h at room temperature, followed by three washes with TBST. DAB chromogenic substrate was used to visualize the expression of the target proteins.

Western blotting. Aliquots of lysate samples containing 30–50 μg of protein were resolved using 10% SDS-PAGE, and electrophoretically transferred onto a polyvinylidene difluoride membrane in a transfer buffer. The membrane was washed with 1× phosphate-buffered saline with Tween-20 three times and incubated with primary antibodies specific for the target proteins. Appropriate secondary antibodies were then added to the membrane prior to incubation for 2 h at room temperature. The proteins on the membrane were visualized using an enhanced chemiluminescence reagent.

Quantitative real-time PCR. Total RNA extraction was performed using Trizol reagent. First-strand cDNA was synthesized using a RevertAid First Strand cDNA synthesis kit in accordance with the manufacturer's protocol. Each sample was analyzed for gene expression via real-time PCR using the SYBR Green Universal PCR master Mix. The specific primers used for VEGF were: forward 5'-TGGCAGAAGAAGGAGACAATAA-3' and reverse 5'-ACGCAGGAAGGCTTGAATAT-3', and those used for actin were forward 5'-ATCGTGCGGGACATCAAGG-3' and reverse 5'-CGGGCAGCTCGTAGCTCTT-3'. The PCR reaction procedure incorporated incubations at 95°C for 5 seconds and 60°C for 30 seconds. For each sample, relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis. PSS19.0 was used for general statistical analysis. Data are represented as means ± S.D. Differences between two groups were assessed *via* Student's *t*-test, and one-way analysis of variance was performed for comparisons of more than two groups. All statistical tests performed were two-tailed, and $p<0.05$ was considered statistically significant.

RESULTS

Appearance of cartilage and the synovium *via* the naked eye

In group A the surface of cartilage of the femur condyles was rough and slightly bright, with some gaps. In group B it was dim, rough, and thin, with even erosion. In group C it was smooth and bright, and oyster white (Fig. 1). In group A the synovium appeared oyster white,

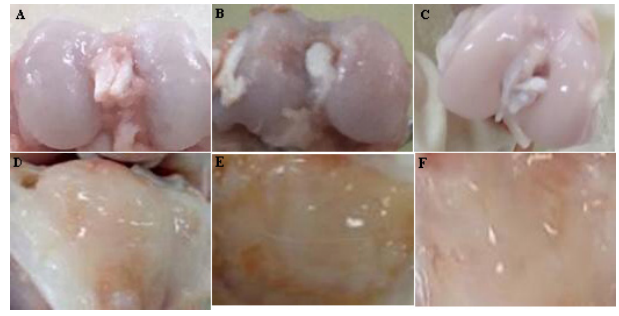

Figure 1. Cartilage morphology (A–C) and synovium morphology (D–F) observed by the naked eye.
A, D: group A; B, E: group B; C, F: group C.

and exhibited a rough surface with a few papillary protrusions. In group B it was light orange colored with some papillary protrusions. In group C it was oyster white, smooth, and bright (Fig. 1).

Appearance of cartilage and the synovium *via* light microscopy

In group A, the thinning of the articular cartilage, slight surface asperity, and partial chondrocyte abolition were evident. Group B exhibited course articular cartilage surfaces, fibrous degeneration, and fissuring. Chondrocyte morphology was nearly normal in group

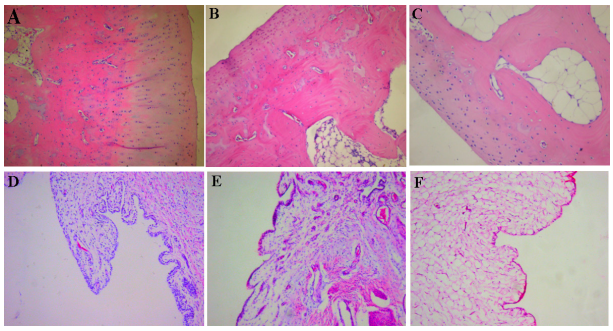

Figure 2. Cartilage morphology (A–C) and synovium morphology (D–F) after hematoxylin eosin staining depicted *via* light microscopy (×100 magnification). A, D: group A; B, E: group B; C, F: group C.

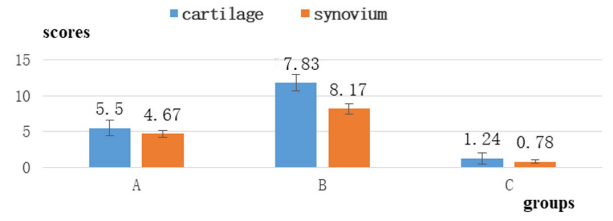

Figure 3. Cartilage morphology determined *via* Mankin scores, and synovial morphology determined *via* Krenn V scores.

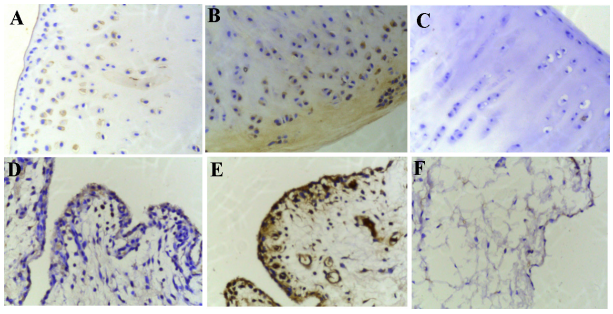

Figure 4. Differences in VEGF expression in chondrocytes (A–C) and synovial cells (D–F) as determined with immunohistochemistry (×400 magnification). A, D: group A; B, E: group B; C, F: group C.

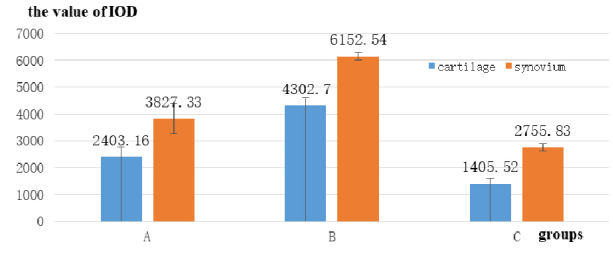

Figure 5. Differences in the IOD scores of immunohistochemically identified VEGF-positive chondrocytes and synovial cells.

C (Fig. 2). Mankin scores based on light microscopy in group A (5.50±1.05) were lower than the scores in group B (7.83±1.17), but higher than the scores in group C (1.24±0.41) (p<0.05; Fig. 3).

In group A there was an increase in the number of inner-layer synovial cells, slight proliferation of synovial cells, some fibrous tissue and vessel generation, and a few infiltrating lymphocytes and monocytes. In group B there was a significant increase in the number of inner-layer synovial cells, a lot of synovial cell proliferation, more fibrous tissue and vessel generation than in group A, and more infiltrating lymphocytes and monocytes than in group A. In group C the inner synovial cells were in a single layer, the arrangement of synovial cells was orderly without fibrous tissue or vessel generation, and there was only sporadic infiltration of lymphocytes and monocytes (Fig. 2). Krenn V scores determined *via* light microscopy were lower in group A (4.67±0.52) than in group B (8.17±0.75), but higher than they were in group C group (0.78±0.24) (p<0.05; Fig. 3).

VEGF expression in cartilage, the synovium, SF, chondrocytes, and synovial cells

In group A the numbers of VEGF-positive chondrocytes and synovial cells were moderate, and in group B the numbers were higher, and the positive cells were stained darker. In group C the numbers of VEGF-positive chondrocytes and synovial cells were low, and the staining of positive cells was light (Fig. 4). In group A the IOD scores of VEGF in chondrocytes (2403.16±366.17) were lower than they were in group B (4302.70±307.80), as was the synovial cell positive expression ratio determined *via* immunohistochemistry (3827.33±563.11 *vs.* 6152.54±6152.54), and in group C both chondrocyte scores (1405.52±194.86) and synovial cell positive expression ratio (2755.83±138.55) were the lowest (p<0.05; Fig. 5).

The protein bands corresponding to VEGF in SF in western blotting indicated a lower VEGF amount in group A than in group B, and the lowest amount in group C (Fig. 6). In real-time PCR, the amount of VEGF mRNA in cartilage in group A (1.73±0.10) was lower than it was in group B (2.69±0.01), and the amount in SF in group A (8.91±1.26) was also lower than in group B (54.65±2.37), but in group C the amounts of VEGF mRNA in both cartilage (1.00±0.01) and SF (1.07±0.08) were the lowest (p<0.05; Fig. 7, Fig. 8).

DISCUSSION

Drug therapies for KOA include nonsteroidal anti-inflammatory drugs and new preparations for inhibiting pathological changes in chondrocytes and the extracellular matrix such as D-glucosamine sulfate, and injectable pain-relieving drugs such as sodium hyaluronate. Regenerative molecules and autologous blood derivatives that can repair tissue have also been used, as have the administration and external application of herbs.

KOA is a disease that mainly affects cartilage, but also invades other joint tissues such as the synovium and subchondral bone, and SF. In addition to progressive degeneration of cartilage, KOA is commonly associated with synovitis (de Lange-Brokaa *et al.*, 2015). However, most studies investigating the pathological changes associated with KOA have focused on cartilage degeneration (including cartilage matrix degradation and chondrocyte apoptosis, among other aspects). Relatively few studies have focused on the relationship between synovial lesions and OA. In the present study, the gross appearance of the synovium was pale orange in the untreated KOA group, and there were many flaky papillary protrusions on the synovial surface. Additionally, synovial tissue sections from that group exhibited an increased number of synovial cell layers (indicating proliferation of synovial cells), disordered arrangement of cells, proliferation of fibrous tissues, and massive angiogenesis, and

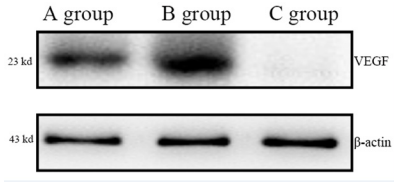

Figure 6. Differences in VEGF protein content in SF as indicated by western blotting.

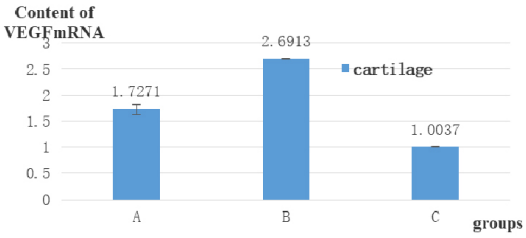

Figure 7. Differences in VEGF mRNA expression in cartilage as determined *via* RT-PCR.

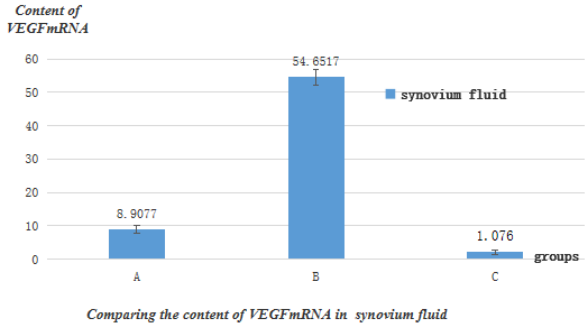

Figure 8. Differences in VEGF mRNA expression in SF as determined *via* RT-PCR.

there was extensive lymphocytic and monocytic infiltration. Pathological changes in the synovium in the untreated KOA group differed significantly from those in the control group.

In the experimental rabbits, 8 weeks after initiation of the OA model, their disease progression was equivalent to the middle stage of human OA. While there should be no acute inflammation at this stage, in the present study the corresponding synovial lesions were substantially changed. With respect to the above, in the early stage of OA attention should be paid to the prevention and treatment of cartilage degeneration while minimizing synovial inflammation. It has been reported that the degree of synovitis was positively correlated with cartilage degeneration (Hill *et al.*, 2007). Previous evidence also indicates that KOA is associated with changes in knee pain that are not related to cartilage loss (de Lange-Brokaar *et al.*, 2016).

However, the majority of previous studies investigating pathological changes and ultrasonic treatment of KOA have focused on effects on cartilage morphology and cell metabolism. No previous report has concentrated on the influence of ultrasound on the morphology of the synovium. Therefore, the present study explored the morphologies of cartilage and the synovium via ultrasound intervention in a model of KOA. In the present study, ultrasound reduced synovial hyperplasia in addition to retarding cartilage degeneration (Figs 1–3). Moreover, the hematoxylin and eosin-derived scores of cartilage and the synovium in the ultrasound group were significantly lower than those in the untreated KOA group ($p<0.05$). Ultrasound also effectively inhibited cartilage degeneration and reduced synovitis in KOA, protecting joints and cartilage. Given these observations, ultrasound may have a therapeutic effect on human KOA. We believe that ultrasound should be recommended as the primary physical therapy modality.

Ultrasound evidently has unique advantages with regard to relieving pain and inflammation as well as delaying cartilage degeneration in KOA patients, but the functional underlying principles involved are less clear. A quantitative imbalance between MMPs and TIMPs is an essential precursor of cartilage degeneration (Tanaka *et al.*, 1998). Reducing MMPs is one of the main approaches to preventing KOA. It has been reported that ultrasound can reduce the expression of MMP-1, 3, 7, and 13 in SF, and inhibit the secretion of nitric oxide. It has also been suggested that low-intensity aggregated ultrasound can reduce the mRNA expression of several MMPs such as MMP-1, 3, and 13 in cartilage and the synovium, as well as elevating TIMP-1 mRNA expression (Xiao *et al.*, 2014).

In the present study, following ultrasonic intervention the numbers of VEGF-positive cells in cartilage and the synovium, the amount of VEGF protein in SF, and the expression of VEGF mRNA in cartilage and SF were lower than they were in the untreated KOA group and higher than they were in the control group, and the difference was statistically significant ($p<0.05$). The regulatory effects of ultrasound on the amounts of VEGF in cartilage, SF, and the synovium have not been reported previously, but prior studies have demonstrated that MMPs are closely related to VEGF. Some researchers have found that increased VEGF promotes the secretion of MMPs, and can also inhibit the degeneration of articular cartilage by inhibiting the synthesis and expression of proteoglycan and type II collagen (Xiao *et al.*, 2014; Chen *et al.*, 2012). It is suggested that VEGF stimulates cartilage cells to increase the production of inflammatory factors and increase the secretion of MMP-1, 3, and particularly 13. Therefore, VEGF is strongly associated with the pathogenesis of KOA, and VEGF can stimulate angiogenesis.

Under normal circumstances VEGF is expressed during articular cartilage growth and ceases at maturity. Studies have shown that the expression of VEGF in cartilage increases with the development of OA. Moreover, VEGF mRNA and protein expression can be detected in the early stage of OA. With regard to mechanism, VEGF stimulates angiogenesis, increases monocyte chemotaxis, vascular permeability, and vasodilatation, and may be positively correlated with osteophyte formation. VEGF is also a major inflammatory mediator of synovial angiogenesis, synovitis, and synovial proliferation. Saetan and others (Saetan *et al.*, 2014) reported that the level of VEGF in SF was positively correlated with the severity of KOA. Ludin and others (Ludin *et al.*, 2013) reported that after synovial VEGF injection there was synovial hyperplasia, calcification of articular cartilage, and osteosclerosis. Additionally, VEGF knockout reduced the development of cartilage, prevented osteoarthritis, and inhibited the tumor necrosis factor-mediated phosphorylation pathway of extracellular regulated protein kinase in chondrocytes (Zhang *et al.*, 2016). In a KOA animal model, anti-VEGF antibody therapy increased the expression of aggregation proteoglycan and type II collagen in articular cartilage cells (Chambers & Matrisian, 1997). In a rabbit model of KOA, the application of anti-VEGF therapy in the early stage reduced

articular cartilage degeneration and osteophyte formation, alleviated synovitis, and reduced pain (Rundhaug, 2005). In the current study, VEGF expression in the synovium, cartilage, and SF, as well as expression of VEGF mRNA in SF and cartilage were all increased in the untreated KOA group compared with the control group, and notably, VEGF expression in the synovium was higher than it was in cartilage (Figs 4, 5, 7, 8), suggesting that VEGF plays an important role in inducing synovial angiogenesis.

The present study suggests that ultrasound may reduce synovitis in rabbits with KOA and delay cartilage degeneration by inhibiting VEGF in cartilage, SF, and the synovium, in turn inhibiting the proliferation of cartilage and synovial vessels. This may reduce the inflammation of cartilage, the synovium, and SF, ultimately resulting in therapeutic effects. And the effects may achieve *via* direct inhibition of VEGF, further studies are needed to clarify whether this occurs *via* a direct pathway.

Competing interests

The authors declare that they have no competing interests.

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