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Effect of LIPUS on inflammatory factors, cell apoptosis and integrin signaling pathway in osteoarthritis animal models

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ABSTRACT

Objective: To study the effect of low-intensity pulsed ultrasound (LIPUS) on inflammatory factors, cell apoptosis and integrin signaling pathway in osteoarthritis animal models. **Methods:** Male New Zealand white rabbits were selected as the experimental animals and randomly divided into sham group, osteoarthritis model group (OA group) and LIPUS intervention group (LIPUS group), animal models with osteoarthritis in hind limb knee joint were established and then given LIPUS intervention. 6 weeks after the intervention, the articular cartilage was separated to detect the expression of inflammatory factors, cell apoptosis molecules and integrin signaling pathway molecules. **Results:** OPN, NO, IL-1 β , TNF- α , Fas, FasL, LC3-II, Beclin-1, Integrin β 1, FAK, ERK1/2, JNK, p38MAPK, MMP-1 and MMP-3 protein expression in articular cartilage of OA group were significantly higher than those of Sham group while Col-I and Col-II protein expression were significantly lower than those of Sham group; OPN, NO, IL-1 β , TNF- α , Fas, FasL, LC3-II, Beclin-1, Integrin β 1, FAK, ERK1/2, JNK, p38MAPK, MMP-1 and MMP-3 protein expression in articular cartilage of LIPUS group were significantly lower than those of OA group while Col-I and Col-II protein expression were significantly higher than those of OA group. **Conclusion:** LIPUS has inhibiting effect on the inflammation, apoptosis and integrin signaling pathway in articular cartilage of osteoarthritis animal models, and it can promote the repair of articular cartilage.

1. Introduction

Osteoarthritis (OA) is a common clinical articular degenerative change, it mainly involves the articular cartilage, and the main characteristics are the destruction and degradation of proteoglycan and collagen in articular cartilage[1]. Under physiological conditions, articular cartilage cell proliferation and apoptosis are in dynamic equilibrium, and they can secrete a variety of proteoglycans and collagens and participate in the formation of extracellular matrix so as to ensure the structural integrity of articular cartilage. In the development and change of OA, under the influence of inflammatory response, apoptosis and other pathological factors, articular cartilage cell proliferation and apoptosis balance is broken, which influences the composition of the extracellular matrix and eventually results in the loss of proteoglycan and collagen in articular cartilage[2,3]. Physical therapy is the preferred therapy for OA. Low-intensity pulsed ultrasound (LIPUS) is physical therapy for osteoarticular diseases developed in recent years, it has promoting effect on osteoblast and chondrocyte proliferation, and it can accelerate fracture end and articular cartilage repair[4]. In the following study, the effect of LIPUS on inflammatory factors, cell apoptosis and integrin signaling pathway in osteoarthritis animal models was analyzed.

2. Experimental materials and experimental methods

2.1 Experimental materials

A total of 27 New Zealand white rabbits were selected as experimental animals, and they were purchased from and fed by Hebei Medical University laboratory animal center. The

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experimental animals were with body mass of 2.5-3.0 kg, male and common level, and they were raised under 24 h diurnal cycles and free to eat and drink. Animal experiments were reviewed by the hospital ethics committee, and the rules were followed for animal experiments and processing after death.

2.2 Experimental reagents and instruments

Protein lysis buffer was from Shanghai Beyotim company, enzyme-linked immunosorbent assay kits were purchased from Shanghai Westang biotechnology company, LIPUS instrument was purchased in Japan Co., LTD, the high-speed refrigerated centrifuge was bought from Jun Bureau instrument company in United States, and microplate reader was purchased from Bio-rad company in United States.

2.3 Experimental methods

2.3.1 Osteoarthritis animal model establishment

Experimental animals were randomly divided into sham group, osteoarthritis model group (OA group) and LIPUS intervention group (LIPUS group), 9 in each group. OA group and LIPUS group were made into animal models of osteoarthritis according to the following method: after ear intravenous injection of 1% pentobarbital sodium for anesthesia, right hind leg knee joint was used as surgical site, medial knee incision was made to separate subcutaneous tissue and muscle, then the joint capsule was opened to expose and cut off anterior cruciate ligament, drawer test was used to confirm the complete ligament rupture, and then the incision was closed. Sham group received anesthesia and joint capsule opening in same method, but the ligament was not cut off, and the incision was directly sutured.

2.3.2 LIPUS intervention

The intervention began after four weeks of model establishment, LIPUS group received intervention by LIPUS instrument, which was as follows: the FREE mode was selected with the frequency set to 3 MHz, intensity to 40 mW/cm^2, 20 min per session and break-make ratio 20%, and they received treatment by LIPUS instrument once a day, 6 d a week, for six weeks of intervention in a row. Sham group and OA group received no special intervention.

2.3.3 Articular cartilage obtaining and detection

Experimental animals were put to death and anatomized to get articular cartilage, it was added in protein lysis buffer and fully grinded, the mixture after grinding was centrifuged in the 4 ℃ centrifuge for 20 min at a speed of 12 000 r/min to separate supernatant, and enzyme-linked immunosorbent assay kits were used to detect OPN, NO, IL-1 β , TNF- α , Fas, FasL, LC3-II, Beclin-1, Integrin β 1, FAK, ERK1/2, JNK, p38MAPK, Col-I, Col-II, MMP-1 and MMP-3 expression.

2.4 Statistical processing

SPSS 18.0 software was used to input the articular cartilage detection data, the comparison of above data among three groups was by variance analysis and $P<0.05$ was the standard of statistical significance in difference.

3. Experimental results

3.1 Inflammatory factor expression in articular cartilage

After 6 weeks of intervention, analysis of inflammatory factors OPN (ng/L), NO (nmol/L), IL-1 β (μg/L) and TNF- α (μg/L) expression in articular cartilage among three groups of experimental animals was as follows: OPN, NO, IL-1 β and TNF- α protein expression in articular cartilage of OA group were significantly higher than those of Sham group, and OPN, NO, IL-1 β and TNF- α protein expression in articular cartilage of LIPUS group were significantly lower than those of OA group. Differences in pair-wise comparison of OPN, NO, IL-1 β and TNF- α protein expression in articular cartilage were statistically significant among three groups of experimental animals ($P<0.05$).

3.2 Cell apoptosis degree in articular cartilage

After 6 weeks of intervention, analysis of cell apoptosis molecules Fas, FasL, LC3-II and Beclin-1 expression in articular cartilage among three groups of experimental animals was as follows: Fas, FasL, LC3-II and Beclin-1 protein expression in articular cartilage of OA group were significantly higher than those of Sham group, and Fas, FasL, LC3-II and Beclin-1 protein expression in articular cartilage of LIPUS group were significantly lower than those of OA group. Differences in pair-wise comparison of Fas, FasL, LC3-II and Beclin-1 protein expression in articular cartilage were statistically significant among three groups of experimental animals ($P<0.05$).

Table 1.

Comparison of inflammatory factor expression in articular cartilage among three groups of experimental animals.

Groups	n	OPN	NO	IL-1 β	TNF- α
Sham group	9	225.64±33.52	68.67±7.07	11.37±1.76	7.59±0.93
OA group	9	649.15±85.58★	176.84±22.34★	32.58±5.52★	24.51±4.26★
LIPUS group	9	385.42±56.48☆	102.15±14.52☆	17.68±2.62☆	10.25±1.77☆

★: comparison between OA group and Sham group, $P<0.05$; ☆: comparison between LIPUS group and OA group, $P<0.05$.

Table 2.

Comparison of cell apoptosis molecule expression in articular cartilage among three groups of experimental animals (ng/L).

Groups	n	Fas	FasL	LC3-II	Beclin-1
Sham group	9	1.03±0.17	1.45±0.20	0.77±0.09	2.31±0.42
OA group	9	2.48±0.52★	3.47±0.62★	2.15±0.42★	8.79±1.04★
LIPUS group	9	1.56±0.25☆	2.21±0.35☆	1.15±0.18☆	3.68±0.66☆

★: comparison between OA group and Sham group, $P<0.05$; ☆: comparison between LIPUS group and OA group, $P<0.05$.

Table 3.

Comparison of integrin signaling pathway molecule expression in articular cartilage among three groups of experimental animals.

Groups	*n*	Integrin β 1	FAK	ERK1/2	JNK	p38MAPK
Sham group	9	2.52±0.35	0.92±0.11	1.34±0.27	0.57±0.08	0.78±0.10
OA group	9	9.41±1.15★	3.46±0.67★	4.46±0.78★	2.16±0.42★	2.31±0.42★
LIPUS group	9	4.18±0.56☆	1.65±0.26☆	2.03±0.36☆	0.98±0.12☆	1.25±0.30☆

★: comparison between OA group and Sham group, $P<0.05$; ☆: comparison between LIPUS group and OA group, $P<0.05$.

Table 4.

Comparison of integrin signaling pathway downstream regulatory molecule expression in articular cartilage among three groups of experimental animals.

Groups	*n*	Col-I	Col-II	MMP-1	MMP-3
Sham group	9	4.85±0.73	6.59±0.94	94.68±11.28	179.57±20.35
OA group	9	1.77±0.31★	2.56±0.42★	264.95±33.64★	426.92±66.38★
LIPUS group	9	3.25±0.56☆	4.77±0.69☆	127.64±17.82☆	282.31±42.62☆

★: comparison between OA group and Sham group, $P<0.05$; ☆: comparison between LIPUS group and OA group, $P<0.05$.

3.3 Integrin signaling pathway function in articular cartilage

After 6 weeks of intervention, analysis of integrin signaling pathway molecules Integrin β 1, FAK, ERK1/2, JNK and p38MAPK expression in articular cartilage among three groups of experimental animals was as follows: Integrin β 1, FAK, ERK1/2, JNK and p38MAPK protein expression in articular cartilage of OA group were significantly higher than those of Sham group, and Integrin β 1, FAK, ERK1/2, JNK and p38MAPK protein expression in articular cartilage of LIPUS group were significantly lower than those of OA group. Differences in pair-wise comparison of Integrin β 1, FAK, ERK1/2, JNK and p38MAPK protein expression in articular cartilage were statistically significant among three groups of experimental animals (P<0.05).

Analysis of integrin signaling pathway downstream regulatory molecules Col-I (μg/L), Col-II (μg/L), MMP-1 (ng/L) and MMP-3 (ng/L) expression in articular cartilage was as follows: Col-I and Col-II protein expression in articular cartilage of OA group were significantly lower than those of Sham group while MMP-1 and MMP-3 protein expression were significantly higher than those of Sham group; Col-I and Col-II protein expression in articular cartilage of LIPUS group were significantly higher than those of OA group while MMP-1 and MMP-3 protein expression were significantly lower than those of OA group. Differences in pair-wise comparison of Col-I, Col-II, MMP-1 and MMP-3 protein expression in articular cartilage were statistically significant among three groups of experimental animals (P<0.05).

4. Discussion

Osteoarthritis is the degenerative disease involving the articular cartilage, extracellular matrix loss in articular cartilage and articular cartilage structure destruction are the basic pathological characteristics of osteoarthritis[5,6], but the mechanism causing changes in articular cartilage structure is not yet clear. Knee joint is the most common part involved by osteoarthritis and can cause pain, stiffness, activity obstacle and other clinical symptoms, which have a negative impact on everyday life. Physical therapy is the most common conservative therapy for clinical treatment of osteoarthritis, including thermal therapy, short-wave therapy, electrical nerve stimulation therapy, ultrasonic therapy, etc. LIPUS is the physical therapy rising in recent years, and the low-frequency ultrasonic wave action on the local tissue could alleviate edema, relieve pain, promote tissue repair and healing, and increase joint mobility[7,8]. In recent years, there is already the study about the positive efficacy of LIPUS treatment of osteoarthritis[9], but it is not yet clear about the molecular mechanism of the LIPUS to improve osseous arthritis disease. In the above study, the molecular mechanism of LIPUS to exert herapeutic effect is analyzed from the perspectives of inflammation, apoptosis and integrin signaling pathway.

Inflammation is an important pathological change of articular cartilage in the development and change of osteoarthritis condition, and the activation of the inflammatory response in articular cartilage and the secretion of various inflammatory factors can promote cartilage destruction. OPN is an extracellular matrix protein with pro-inflammatory activity, and it can increase the expression and secretion of a variety of inflammatory mediators in articular cartilage[10]; NO is a gas molecule that has a variety of biological activities, it can participate in prostaglandin synthesis and increase inflammation in articular cartilage, and it can also enhance the damage of IL-1 β , TNF- α and other inflammaotory mediators to the articular cartilage; IL-1 β and TNF- α participate in the regulation of the inflammatory response, the destruction of the cartilage cells and the change of extracellular matrix in the articular cartilage together[11]. In the study, it was observed after animal model of osteoarthritis was established that OPN, NO, IL-1 β and TNF- α protein expression in articular cartilage of OA group significantly increased. This means that the activation of the inflammatory response is closely related to the occurrence of osteoarthritis. Further analysis of the regulating effect of LIPUS on inflammatory factors in articular cartilage showed that OPN, NO, IL-1 β and TNF- α protein expression in articular cartilage of LIPUS group were significantly lower than those of OA group. This means that LIPUS has significant inhibitory effect on the inflammation in osteoarthritis progression.

The activation of the inflammatory response in articular cartilage can start the cell apoptosis to cause local tissue damage. Fas/FasL is apoptosis pathway is the apoptosis pathway closely related to the inflammatory response, and high Fas expression caused by inflammation can pass down death signals through FasL receptors,

and cause apoptosis through the cascade activation of a variety of downstream Caspase[12]. Autophagy is another cell apoptosis pathway associated with inflammation, the excessive activation of the inflammatory response in articular cartilage can lead to excessive autophagy activation, and then result in cell apoptosis[13]. LC3 plays a key role in the process of cell autophagy, precursor LC3 is processed into LC3-I, LC3-I will further split into LC3-II, and the LC3-II has the activity to mediating autophagy; Beclin-1 is a marker gene of autophagy in mammals and involved in the formation of autophagosome[14]. In the study, analysis of the apoptosis molecule expression in articular cartilage of osteoarthritis animal models showed that Fas, FasL, LC3-II and Beclin-1 protein expression in articular cartilage of OA group increased significantly. This means that Fas/FasL apoptosis pathway and autophagy apoptosis pathway are closely related to the occurrence of osteoarthritis. Further analysis of the regulating effect of LIPUS on apoptosis molecules in articular cartilage showed that Fas, FasL, LC3-II and Beclin-1 protein expression in articular cartilage of LIPUS group were significantly lower than those of OA group. It means that LIPUS has significant inhibitory effect on the Fas/FasL apoptosis pathway and autophagy apoptosis pathway in osteoarthritis progression.

Articular cartilage cells will be damaged under the influence of inflammatory response, apoptosis and other pathological factors, which will affect the cartilage cells to synthesize and secrete proteoglycan and collagen, and thus lead to the extracellular matrix component loss in articular cartilage and the articular cartilage degradation. Integrin β 1 signaling pathway is an important pathway adjusting the extracellular matrix degradation in articular cartilage, and Integrin β 1 can cause the FAK phosphorylation, then activate downstream signaling molecules ERK1/2, JNK and p38MAPK and regulate the expression of a variety of target genes[15,16]. In the study, analysis of the Integrin β 1 signaling pathway function showed that Integrin β 1, FAK, ERK1/2, JNK and p38MAPK protein expression in articular cartilage of OA group increased significantly, and LIPUS could inhibit the Integrin β 1, FAK, ERK1/2, JNK and p38MAPK protein expression in articular cartilage. The change of Integrin β 1 signaling pathway function can affect the expression of downstream MMP-1 and MMP-3 to cause the collagen ingredients Col-I and Col-II degradation in articular cartilage[17,18]. In the study, analysis of the contents of above integrin signaling pathway downstream regulatory molecules in articular cartilage showed that MMP-1 and MMP-3 expression in articular cartilage of OA group significantly increased while Col-I and Col-II expression significantly decreased; LIPUS could inhibit the MMP-1 and MMP-3 expression and increase the Col-I and Col-II expression in articular cartilage. This means that LIPUS has inhibitory effect on integrin signaling pathways in osteoarthritis progression.

Based on the results of above animal experiments, it can be concluded that LIPUS can significantly improve the condition of osteoarthritis animal models, and the inflammatory response, apoptosis and integrin signaling pathways in articular cartilage are all inhibited by the LIPUS; LIPUS can promote the repair of articular cartilage by above change.

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