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Original Article

Therapeutic effect of α-lipoic acid on osteoarthritis patients and its influence on TLR4/NF-κB and IL-23/IL-17 signaling pathway

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Abstract: Objective: To investigate the therapeutic effect of α-lipoic acid on osteoarthritis and its effects on toll-like receptor-4/nuclear factors-κB (TLR4/NF-κB) and IL-23/IL-17 signaling pathways. Methods: Seventy-eight patients with osteoarthritis in the knee were selected for a prospective study and were randomly divided into the control group and the α-lipoic acid group. Patients in the control group took ibuprofen tablets and patients in the α-lipoic acid group took α-lipoic acid tablets once a day orally for 4 weeks. Before and after treatment, visual analogue scale (VAS) and Western Ontario and McMaster University Osteoarthritis Index (WOMAC) scores were determined. The levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-17 (IL-17) and interleukin-23 (IL-23) in serum were measured using enzyme-linked immunosorbent assay. After treatment, mononuclear cells in the venous blood of the patients were subjected to Western blot detection of TLR4 and NF-κB. Results: After treatment, the VAS pain score and WOMAC function score of the patients in the α-lipoic acid group were significantly lower than those in the control group (both $P<0.01$). The levels of IL-1β ($P<0.001$), IL-6 ($P<0.05$), TNF-α ($P<0.001$), IL-17 ($P<0.001$), and IL-23 ($P<0.01$) in the serum of patients treated with α-lipoic acid after treatment were significantly lower than those in the control group. Alpha-lipoic acid reduced the expression of TLR4 and NF-κB in the peripheral blood mononuclear cells (both $P<0.001$). Conclusion: Alpha-lipoic acid can improve the clinical symptoms of the patients with osteoarthritis and reduce the level of inflammation, which may be associated to the inhibition of TLR4/NF-κB and IL-23/IL-17 signaling pathways.

Keywords: α-lipoic acid, osteoarthritis, visual analogue scale, Western Ontario and McMaster University Osteoarthritis Index, inflammatory factors

Introduction

Osteoarthritis is a disease with joint pain and movement restriction as the main clinical manifestations which particularly occurs in the knees. This may be caused by several factors such as age, obesity, trauma, weight-bearing, and hyperactivity. The main pathological manifestations included damage in the knee joint cartilage, subchondral sclerosis, and narrowing of joint space [1, 2]. Persistent chronic inflammation is closely related to the severity of osteoarthritis, manifested as synovial infiltration of inflammatory cells such as macrophages, mast cells, and T lymphocytes, and continuous secretion of inflammation factors such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) [3]. It has been found that toll-like receptor-4 (TLR4) can recognize endogenous ligand molecules and activate downstream nuclear factors-κB (NF-κB)-mediated signaling pathways. This allows immune cells to secrete a substantial amount of IL-23 to further enhance the secretion of interleukin-17 (IL-17), thereby promoting chemotaxis, proliferation, and immune system activation [4]. Therefore, TLR4/NF-κB and interleukin-23 (IL-23)/IL-17 signaling pathways can play the important roles for the amplification of inflammatory response, and the persistence of osteoarthritis.

Alpha-lipoic acid is a low molecular weight antioxidant synthesized by the octanoate conversion in the mitochondria. It inhibits the release of reactive oxygen species and promote the

Table 1. Comparison of general data between two groups

General data	Control group (n=39)	α-lipoic acid group (n=39)	t/χ^2	P
Age (year)	30.44±8.57	31.62±7.90	0.632	0.529
Gender			0.269	0.604
Male (n, %)	28 (71.79)	30 (76.92)		
Female (n, %)	11 (28.21)	9 (23.08)		
Disease course (month)	18.35±7.52	19.86±8.71	0.820	0.415

production of endogenous antioxidant molecules such as glutathione and vitamin E [5, 6]. In addition, α-lipoic acid also has an inflammatory inhibitory effect and a better therapeutic effect in diseases such as ulcerative colitis, nephritis, and thyroiditis [7-9]. Wang et al. have found that α-lipoic acid can prevent the induction of endoplasmic reticulum stress and NF-κB signaling pathway; it can reduce the secretion of TNF-α and relieve the degeneration of articular cartilage in a rat model with osteoarthritis induced by sodium iodoacetate. This indicates that α-lipoic acid plays a protective role in osteoarthritis [10]. Nevertheless, most studies on the treatment of osteoarthritis with α-lipoic acid remain in the cell and animal experiments, and the relevant clinical evidence is still lacking.

Therefore, this study selected 78 patients with knee osteoarthritis to conduct a prospective study to observe the effect of α-lipoic acid treatment on the curative effect, especially its impact on the visual analogue scale (VAS) pain score and Western Ontario and McMaster University Osteoarthritis Index (WOMAC) function score and its effects on TLR4/NF-κB and IL-23/IL-17 signaling pathways.

Materials and methods

Clinical data

Seventy-eight patients with knee osteoarthritis admitted in Dezhou People's Hospital from June 2015 to December 2017 were selected for this prospective cohort study. They were randomly divided into the control group (n=39) and the α-lipoic acid group (n=39).

Diagnosis criteria for knee osteoarthritis: Signs and symptoms of knee swelling, tenderness, friction fremitus, knee pain, activity limitation; narrowing of joint space, subchondral sclerosis and other relevant imaging findings [11].

Exclusion criteria: Patients with other conditions such as liver and kidney dysfunction, drug allergies, rheumatic immune diseases, hematological diseases, and skin diseases.

General data of the two groups were collected and compared, such as age, gender, course of disease and other basic demographic information. Informed consent was signed by all selected patients and the study was approved by the Ethics Committee of Dezhou People's Hospital.

Therapeutic regimen

The control group took ibuprofen tablets (Sino-US Tianjin Glaxo-SmithKline Pharmaceutical Co., Ltd., China) orally, once a day for 4 weeks, with a dosage of 0.3 g [12]. The α-lipoic acid group took α-lipoic acid tablets (Shandong Qidu Pharmaceutical Co., Ltd., China) orally, once a day for 4 weeks with a dosage of 0.6 g [13].

Observation indicators

Primary observation indicators: All patients were subjected to the determination of VAS pain scoring and WOMAC function scoring before and after treatment [11]. VAS pain scoring is a self-based rating scale of the degree of pain using a range of 0-10 with 0 as painless and 10 as severe pain. Ranging from low to high, the pain gradually increased. WOMAC function scoring is based on a total of 24 indicators in terms of joint pain (5 indicators), stiffness (2 indicators) and the difficulty of daily activities (17 indicators), with a classification of mild having <80 points, moderate having 80-120 points, and severe having >120 points.

Secondary observation indicators: Venous blood was collected from all patients before and after treatment to determine the concentration levels of IL-1β (NeoBioscience, China; EHC0-02b.96), IL-6 (Millipore, USA; EZHIL6), TNF-α (NeoBioscience, China; EHC103a.96), IL-17 (NeoBioscience, China; EHC170.96), and IL-23 (NeoBioscience, China; EHC171.96) in the serum which were measured by using enzyme-linked immunosorbent assay.

Table 2. Comparison of VAS pain scores before and after treatment in two groups

VAS pain score	Control group	α-lipoic acid group	t	P
Pre-treatment	6.25±1.66	6.55±1.70	0.789	0.433
Post-treatment	4.14±1.21	3.20±1.37	3.212	0.002
t	6.415	9.582		
P	<0.001	<0.001		

Note: VAS, visual analogue scale.

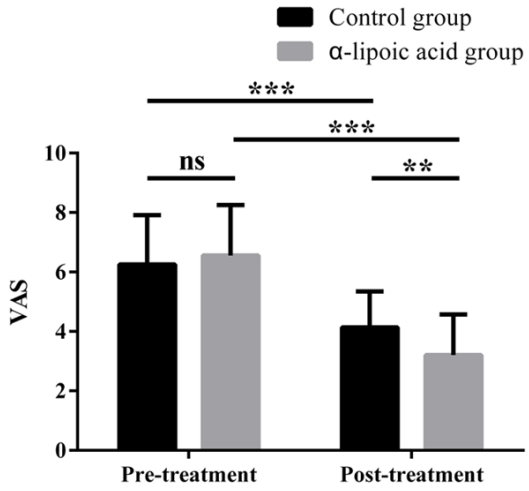

Figure 1. Comparison of VAS pain scores before and after treatment in two groups. ^{ns}P>0.05, **P<0.01, ***P<0.001. VAS, visual analogue scale.

Western blotting (WB)

After treatment, WB detection of TLR4 and NF-κB was performed on the venous blood mononuclear cells of all patients. This specific method was followed as: peripheral blood mononuclear cells from two groups of patients were isolated using a human peripheral blood mononuclear cell separating medium (Solarbio, China; P8610), and 10^6 cells were lysed by RIPA lysate (Beyotime, China; P0013), followed by BCA protein quantification (Beyotime, China; P0012). Approximately, 20 μg of total protein was transferred to PVDF membrane after polyacrylamide gel electrophoresis, blocked for 2 hours by 5% bovine serum albumin, and incubated overnight using anti-TLR4 antibody (1:1,000; Abcam, USA; ab13556), anti-NF-κB antibody (1:1,000; Abcam, USA; ab32360), and anti-GAPDH antibody (1:5,000; Abcam, USA; ab9485). After washing for three times, the protein was incubated for 1 hour using a goat anti-rabbit second antibody (1:2,000; Boster, China; BA1056) labeled with horseradish peroxidase. After washing for three more times, the color was developed with ECL developing solution (Beyotime, China; P0018), and the electrochemical gel imager (Bio-Rad ChemiDoc XRS, USA) was used for exposure and photographing. The ratio of target protein grey value to GAPDH grey value in the control group was taken as 1 [14].

Statistical analysis

Statistical analysis was performed using SPSS 20.0. All the measurement data conform to the normal distribution and were expressed as mean±standard deviation ($\bar{x}$±sd). t test was used to compare the measurement data between the two groups in accordance with normal distribution. All the counting data were represented as patient cases and percentages, and the comparison between the two groups was conducted using the χ^2 test. P<0.05 is used as the test standard.

Results

Comparison of general data between two groups

The results showed that there were no statistical differences in the age, gender, and disease course (all P>0.05) between the control group and the α-lipoic acid group and thus, the patients were comparable. See **Table 1**.

Comparison of VAS pain scores before and after treatment in two groups

The results showed that there was no significant difference in the VAS pain scores before treatment between the two groups (P>0.05). The VAS pain scores after treatment were significantly lower in both groups than before treatment (P<0.001); and patients treated with α-lipoic acid had significantly lower VAS pain scores after treatment than those in the control group (P<0.01). See **Table 2** and **Figure 1**.

Comparison of WOMAC function scores before and after treatment in two groups

The results showed that there was no significant difference in the WOMAC function scores

Table 3. Comparison of WOMAC function scores before and after treatment in two groups

WOMAC score	Control group	α-lipoic acid group	t	P
Pre-treatment	40.09±11.37	42.46±12.05	0.893	0.375
Post-treatment	28.31±9.05	21.37±8.52	3.487	0.001
t	5.062	8.925		
P	<0.001	<0.001		

Note: WOMAC, Western Ontario and McMaster University Osteoarthritis Index.

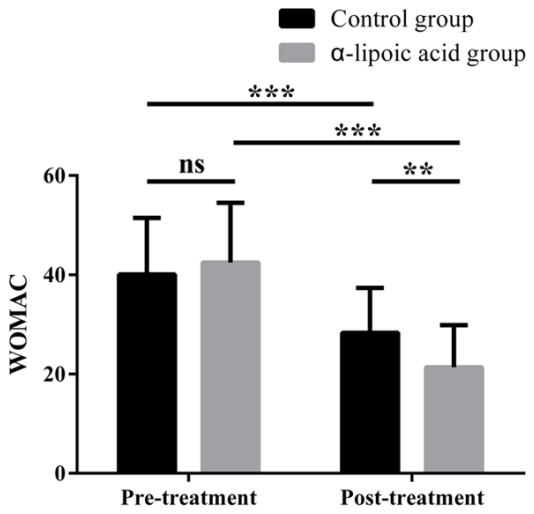

Figure 2. Comparison of WOMAC function scores before and after treatment in two groups. ^{ns}P>0.05, **P<0.01, ***P<0.001. WOMAC, Western Ontario and McMaster University Osteoarthritis Index.

before treatment between the two groups (P>0.05). The WOMAC function scores in both groups were significantly reduced after treatment compared with the scores before treatment (P<0.001). The WOMAC function scores in the α-lipoic acid group were also significantly lower than those in the control group (P<0.01). See **Table 3** and **Figure 2**.

Comparison of serum inflammatory factors before and after treatment in two groups

The results showed that there were no significant differences in serum levels of IL-1β, IL-6, and TNF-α between the two groups before treatment (all P>0.05). The levels of IL-1β and TNF-α in the serum of the control group were significantly lower after treatment (both P<0.001), but no significant difference was seen in the levels of IL-6 before and after treatment (P>0.05). In comparison with the pre-treatment levels, the serum levels of IL-1β, IL-6, and TNF-α in patients treated with α-lipoic acid were significantly lower (all P<0.001). After treatment, levels of IL-1β (P<0.001), IL-6 (P<0.05), and TNF-α (P< 0.001) in the serum of α-lipoic acid group were all lower than those of the control group. See **Table 4** and **Figure 3**.

Comparison of serum IL-17 and IL-23 levels before and after treatment in two groups

The results showed that there were no significant differences in the serum levels of IL-17 and IL-23 between the two groups before treatment (both P>0.05); in comparison with the pre-treatment levels, serum IL-17 level in the control group decreased after treatment (P<0.05), but IL-23 level did not significantly change (P>0.05). The levels of IL-17 and IL-23 in the serum of the patients in the α-lipoic acid group were both significantly reduced compared to the levels before treatment (both P<0.001); After treatment, the levels of IL-17 (P<0.001) and IL-23 (P<0.01) in the serum of patients treated with α-lipoic acid were both significantly lower than those of the control group. See **Table 5** and **Figure 4**.

Comparison of expression of TLR4 and NF-κB in peripheral blood mononuclear cells after treatment in two groups

The results showed that the expression levels of TLR4 and NF-κB in the peripheral blood mononuclear cells of patients in the α-lipoic acid group were significantly lower than those in the control group (both P<0.001). See **Figure 5**.

Discussion

IL-1β, IL-6, TNF-α and other inflammatory factors can stimulate chondrocytes to secrete matrix metalloproteinases, strengthen the bone matrix catabolism, induce the absorption of bone matrix, and play an important role in the pathological progression of knee osteoarthritis [15, 16]. At present, the main drugs for osteoarthritis treatment are glucocorticoids, non-steroidal anti-inflammatory drugs, and immunosuppressants. However, the clinical effects are not satisfactory. Anti-inflammatory drugs such as ibuprofen, dexamethasone and methotrexate may even promote osteoclast

Table 4. Comparison of serum inflammatory factors before and after treatment in two groups

Inflammatory factor	Control group	α-lipoic acid group	t/χ^2	P
IL-1β (ng/L)				
Pre-treatment	105.41±19.44	101.08±19.27	0.988	0.326
Post-treatment	89.50±13.36	62.74±10.69	9.767	<0.001
t	4.212	10.870		
P	<0.001	<0.001		
IL-6 (ng/L)				
Pre-treatment	186.24±31.15	194.01±33.60	1.059	0.293
Post-treatment	179.48±30.02	164.19±28.72	2.298	0.024
t	0.976	4.213		
P	0.332	<0.001		
TNF-α (ng/L)				
Pre-treatment	21.58±4.43	20.11±4.20	1.504	0.137
Post-treatment	16.32±3.76	11.41±3.18	6.227	<0.001
t	5.653	10.310		
P	<0.001	<0.001		

Note: IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

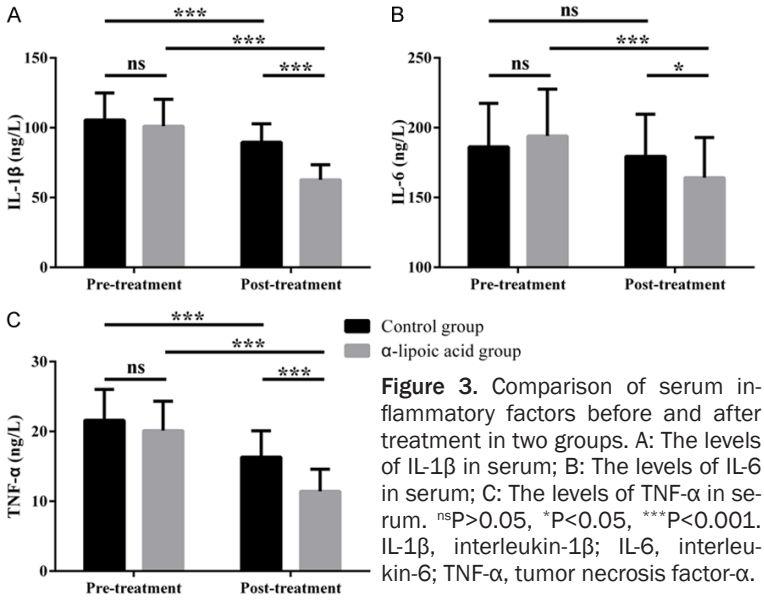

Figure 3. Comparison of serum inflammatory factors before and after treatment in two groups. A: The levels of IL-1β in serum; B: The levels of IL-6 in serum; C: The levels of TNF-α in serum. $^{ns}P>0.05$, $^{*}P<0.05$, $^{***}P<0.001$. IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

production and aggravate bone destruction [17]. Therefore, it is of great clinical significance to find safer and more effective therapeutic drugs.

Recent studies have found that α-lipoic acid not only has antioxidant effects, but also has significant inhibitory effects on inflammation [18, 19]. For example, Zhang et al. have found that α-lipoic acid inhibits lipopolysaccharide-induced monocyte activation, prevents the activation of NF-kB signaling pathway, and reduces the secretion of TNF-α and other inflammatory factors in the lung, heart, aorta and other tissues [20].

At present, in vitro cytology experiments and reports on animal models have confirmed that α-lipoic acid also has a protective effect on osteoarthritis. For instance, Sun et al. have found that α-lipoic acid can inhibit IL-1β from inducing chondrocyte to secrete Matrix metalloproteinase-13, and Wang et al. have found that α-lipoic acid can relieve cartilage degeneration in a rat model of osteoarthritis [10, 21]. However, the therapeutic effect of α-lipoic acid on osteoarthritis still lacks relevant clinical evidence.

Therefore, 78 patients with osteoarthritis of the knee were selected in this study for a prospective cohort study. After a 4-week treatment, the VAS pain score and WOMAC function score of the patients in the α-lipoic acid group were significantly lower than those in the control group. This indicates that the therapeutic effect of α-lipoic acid on osteoarthritis is better than that of ibuprofen. This phenomenon may be due to the fact that α-lipoic acid has complex antioxidant and anti-inflammatory effects while ibuprofen only has better therapeutic effects in terms of anti-inflammation and analgesia. In addition, the serum levels of IL-1β, IL-6, and TNF-α after treatment were all lower in the α-lipoic acid group than those in the control group. This indicates that α-lipoic acid has stronger inflammatory inhibition on osteoarthritis than non-steroidal anti-inflammatory drugs such as ibuprofen.

IL-17 and its upstream regulatory factor IL-23 can promote the secretion of inflammatory factors such as IL-1β, IL-6 and TNF-α and can increase the pathological progress of osteoar-

Table 5. Comparison of serum IL-17 and IL-23 levels before and after treatment in two groups

Inflammatory factor	Control group	α-lipoic acid group	t/χ^2	P
IL-17 (ng/L)				
Pre-treatment	35.17±6.85	34.86±6.74	0.202	0.841
Post-treatment	31.81±5.64	22.60±4.22	8.165	<0.001
t	2.365	9.628		
P	0.021	<0.001		
IL-23 (ng/L)				
Pre-treatment	27.15±5.67	28.42±5.98	0.962	0.339
Post-treatment	25.83±5.08	22.11±4.89	3.295	0.002
t	1.083	5.101		
P	0.282	<0.001		

Note: IL-17, interleukin-17; IL-23, interleukin-23.

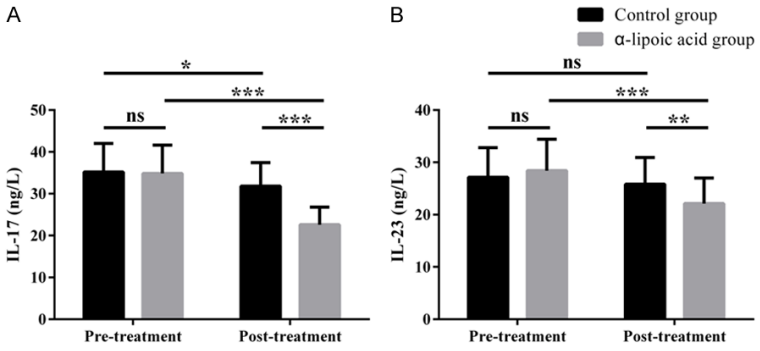

Figure 4. Comparison of serum IL-17 and IL-23 levels before and after treatment in two groups. A: The levels of IL-17 in serum; B: The levels of IL-23 in serum. $^{ns}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. IL-17, interleukin-17; IL-23, interleukin-23.

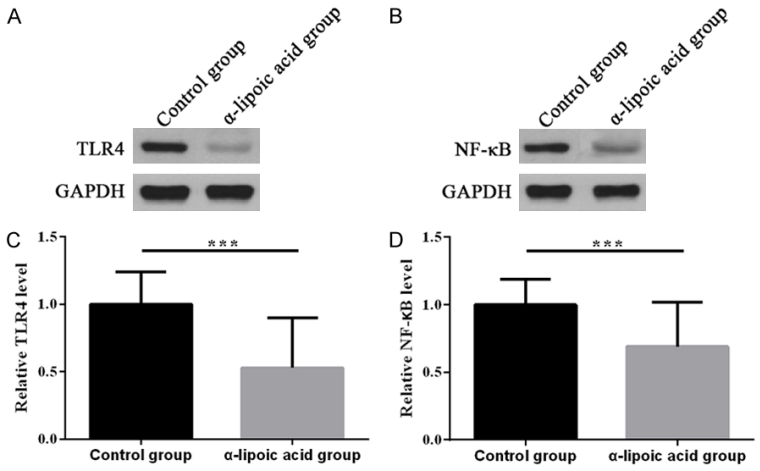

Figure 5. Comparison of expression of TLR4 and NF-κB in peripheral blood mononuclear cells after treatment in two groups. A: The representative image of TLR4 expression detected by WB; B: The representative image of NF-κB expression detected by WB; C: Comparison of relative grayscale value of TLR4; D: Comparison of relative grayscale value of NF-κB. $^{***}P<0.001$. TLR4, toll-like receptor-4; NF-κB, nuclear factors-κB; WB, western blot.

thritis. For example, Askari et al. found that the serum levels of IL-17 and IL-23 in patients with osteoarthritis were significantly higher than those of normal volunteers, and the degree of increase was correlated with the WOMAC function score [22]. Although the upstream regulatory mechanism of IL-23/IL-17 has not been clearly elucidated, a substantial number of studies suggest that the activation of IL-23/IL-17 signaling pathway is dependent on the regulation of TLR4/NF-κB. Yan et al. found that TLR4 induces neutrophil infiltration through the activation of the IL-23/IL-17 signaling pathway [23]. Cho et al. have found that the activation of IL-23/IL-17 signaling pathway in $CD4^+$ T cells depends on NF-κB regulation [24].

In summary, TLR4/NF-κB and IL-23/IL-17 signaling pathways may play an essential role in the maintenance and deterioration of inflammation in osteoarthritis as an inflammatory amplifier. Therefore, it is essential to investigate the effects of α-lipoic acid on TLR4/NF-κB and IL-23/IL-17 signaling pathways. The results of this study show that levels of IL-23 and IL-17 in serum and expression of TLR4 and NF-κB in peripheral blood mononuclear cells in patients treated with α-lipoic acid are lower compared with the control group, indicating that α-lipoic acid can effectively inhibit the activation of TLR4/NF-κB and IL-23/IL-17 signaling pathways in osteoarthritis.

Although this paper studied the therapeutic effect of α-lipoic acid on patients with osteoarthritis and investigat-

ed the TLR4/NF-κB and IL-23/IL-17 signaling pathways, there are still many deficiencies and limitations. 1) As the observation time was relatively short (only 4 weeks), further studies are still needed to confirm whether α-lipoic acid still has protective effects in the long-term prognosis of osteoarthritis. 2) This article studied the effect of α-lipoic acid treatment on TLR4/NF-κB and IL-23/IL-17 signaling pathways, but there was a lack of direct evidence to determine the involvement of other related inflammatory pathways in the therapeutic effects of α-lipoic acid. 3) The present study found that the excessive secretion of matrix metalloproteinases can degrade the extracellular matrix of cartilage and cause the retrogression of articular cartilage in patients with osteoarthritis, but studies are still lacking to verify the effects of α-lipoic acid on the secretion of matrix metalloproteinase in chondrocytes [25, 26].

In conclusion, α-lipoic acid can improve the clinical symptoms and reduce the inflammatory processes in patients with osteoarthritis, which may be related to the inhibition of TLR4/NF-κB and IL-23/IL-17 signaling pathways. Further studies on the effect of α-lipoic acid on the long-term prognosis of patients with osteoarthritis are still needed. In addition, in vitro models of chondrocytes, RNA interference and other techniques may be used to specifically knockdown the expression of TLR4, NF-κB and other cytokines, which can provide direct evidence for the role of α-lipoic acid in the TLR4/NF-κB and IL-23/IL-17 signaling pathways.

Disclosure of conflict of interest

None.

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