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Original Article

Effect of Etoricoxib on miR-214 and inflammatory reaction in knee osteoarthritis patients

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Abstract: Purpose: To explore the effect of Etoricoxib on serum miR-214 expression level and inflammatory reaction in patients with knee osteoarthritis. Methods: 96 patients with knee osteoarthritis admitted to our hospital (January 2019 to January 2020) were selected. 48 patients in the control group received Celecoxib and 48 patients in the observation group received Etoricoxib. The treatment effect, knee function, inflammatory factor level, immune function, and serum miR-214 expression level of the two groups were compared. 6 months after treatment, the incidence of complications (deformities, deep infections and severe pain) between the two groups was compared. Results: (1) The observation group had a higher total effective rate (93.75%) in comparison to the control group (72.92%) ($P<0.05$). (2) Before treatment, the serum miR-214 expression level of the two groups was basically the same ($P>0.05$). After treatment, the serum miR-214 expression level of the two groups decreased significantly, with a more marked decrease in the observation group ($P<0.05$). (3) Before treatment, the levels of IL-1β, TNF-α, and hs-CRP were not statistically different in the two groups ($P>0.05$). After treatment, IL-1β, TNF-α, and hs-CRP in both groups decreased, and the decrease in the observation group was significantly greater ($P<0.05$). (4) Before treatment, the levels of $CD3^+CD8^+$ and $CD3^+$ were basically the same in both groups ($P>0.05$). After treatment, the levels of $CD3^+CD8^+$ and $CD3^+$ in the two groups increased, and for the observation group, were significantly greater $P<0.05$. (5) The Lysholm score was higher in the observation group than it was in the control group (inter-group effect: $F = 58.070$, $P<0.001$), and the Lysholm score of both groups tended to increase with time (time effect: $F = 145.900$, $P<0.001$). Grouping and time showed an interactive effect (interactive effect: $F = 8.646$, $P<0.001$). 6 months after treatment, observation group showed a lower complication rate when compared to the control groupt ($P<0.05$). Conclusion: Etoricoxib has a strong effect on patients with knee osteoarthritis. It can significantly reduce the expression of serum miR-214 and the level of inflammatory factors, and is worthy of clinical application.

Keywords: Etoricoxib, knee osteoarthritis, miR-214, inflammatory reaction

Introduction

Knee osteoarthritis, as a chronic disease, is a bone and joint disease caused by degenerative hyperplasia of articular cartilage, and is manifested as joint swelling or pain and movement disorders [1, 2]. Knee osteoarthritis features a long disease course and its prevalence increases with age, which remains one of the leading causes for disability in the elderly. Currently, non-steroidal anti-inflammatory drugs are predominantly used as first-line of clinical treatment for knee osteoarthritis. As one of non-steroidal anti-inflammatory drugs, Celecoxib plays a role in relieving pain symptoms and delaying the progression of disease [3]. Etoricoxib is a new type of highly selective cyclooxygenase-2 (COX-2) inhibitor, and bears anti-inflammatory and analgesic effects [4]. Inflammation is considered a risk factor that promotes the progression of osteoarthritis. It is closely related to cartilage loss and the clinical symptoms of osteoarthritis, including joint pain, swelling, stiffness, and inflammation indicators. Of these inflammatory mediators, interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), are included. Osteoarthritis chondrocytes produce a variety of matrix-degrading enzymes during stress and inflammation. These degrading enzymes are dysregulated in the osteoarthritis chondrocytes. The degradation of cartilage during development of osteoarthri-

Table 1. Comparison of general data between two groups

Group	Sex [n (%)]		Age	Disease course	BMI
	Male	Female	($\bar{x}$ ± s, years)	($\bar{x}$ ± s, months)	($\bar{x}$ ± s, kg·m^2)
Control group (n = 48)	22 (45.83)	26 (54.17)	61.95±4.54	22.67±4.12	22.79±3.24
Observation group (n = 48)	17 (35.42)	31 (64.58)	62.48±4.79	23.06±4.09	22.86±3.39
χ^2/t	1.080		0.556	0.465	0.103
P	0.299		0.579	0.643	0.918

tis is attributable to the increase and abnormality of their expression and activity. As the signaling pathways involved in inflammation and biomechanical stress are similar, these pathways may also induce and amplify the expression of cytokine and chemokine genes. Therefore, inflammatory mediators are regulators of osteoarthritic cartilage damage and repair mechanism defects. miRNA is a short endogenous non-coding RNA that regulates protein expression by inhibiting the translation of mRNA, and miRNA is assumed to be involved in the pathogenesis of a variety of inflammatory and autoimmune diseases, and the development of osteoarthritis as well. In recent years, more miRNAs have been proven to play an important role in orthopedic diseases, and miRNA-214 can regulate the function of osteoclasts in the process of osteoporotic bone remodeling. Therefore, the present study observed the effect of Etoricoxib on the expression level of serum miR-214 and inflammation reaction in patients with knee osteoarthritis, aiming to provide a reference for clinical treatment of knee osteoarthritis.

Subjects and material

General data

Patients with knee osteoarthritis admitted to our hospital (January 2019 to January 2020) were enrolled in this study. Inclusion criteria: ① patients met the diagnostic criteria for knee osteoarthritis [5]; ② no allergy to the relevant drugs in this study; ③ patients agreed to this study and provided consent form. Exclusion criteria: ① other joint diseases; ② autoimmune diseases and malignant tumors; ③ serious liver, kidney, heart and other important organ diseases; ④ mental disorders; ⑤ female patients who were lactating or pregnant; ⑥ patients had used analgesics, drugs for treating osteoarthritis, and similar drugs such as Etoricoxib in the past 30 days. 96 patients were finally included, and were divided into a control group (n = 48) and an observation group (n = 48) using a random number table method. The general data were not statistically different in the two groups in gender, age, course of disease, body mass index, etc. (P>0.05, **Table 1**). Approval was obtained from the ethics committee of our hospital.

Methods

The control group was given Celecoxib capsules (produced by Pfizer Pharmaceuticals Co., Ltd.; SFDA approval number: J20140072; specifications: 200 mg × 6 s), oral administration, 200 mg/time and 2 times/day.

The observation group received Etoricoxib (produced by Frosst Iberica SA; SFDA approval number: J20180057; specifications: 120 mg × 5 s), 120 mg/time, 1 time/day. Both groups were treated for 3 months.

Serum miR-214 expression level testing method: (1) The cartilage and subchondral tissue blocks were isolated: ① the specimen was aseptically taken and stored at -80°C; ② the specimen was placed in a container containing liquid nitrogen and fixed using surgical forceps; micro-power magic drill was applied to cut and separate the tissue blocks to obtain articular cartilage and subchondral tissue blocks. (2) The expression level of miR-214 was tested: ① the tissue was placed in a mortar, quickly ground to a powder in a liquid nitrogen environment, and put into a 1.5 ml EP tube; ② 1 ml Trizol was added to the sample EP tube, shaken uniformly, letting it stand for about 15 min at room temperature; ③ chloroform (Zhejiang Deyer Pharmaceutical Co., Ltd. No. 175001) was added to the sample EP tube in the amount of Trizol: chloroform = 1 ml: 0.2 ml, shake for 15 s, letting it stand at room temperature for about 15 min and centrifuging at 4°C and 12000 rpm for 15 min; ④ the upper

Table 2. Comparison of treatment effect between two groups [n (%)]

Group	Uncured	Improved	Cured	Total effective rate
Control group (n = 48)	13 (27.08)	23 (47.92)	12 (25.00)	35 (72.92)
Observation group (n = 48)	3 (6.25)	15 (31.25)	30 (62.50)	45 (93.75)
χ^2				6.075
P				0.014

aqueous phase was drawn into a 0.5 ml EP tube; 0.5 ml of isopropanol was added, and inverted repeatedly to mix evenly, letting it stand for about 10 min (to fully precipitate RNA), centrifuging at 4°C and 12000 rpm for 5 min; ⑤ the supernatant was removed, 1 ml of 75% alcohol was added, and it was centrifuged at 4°C and 12,000 rpm for 5 min; ⑥ the supernatant was removed again, dried for about 15 min, DEPC water was added to dissolve (25 μl/tube, about 20~25 min), and shaken uniformly.

Determination of $CD3^+CD8^+$ lymphocytes and $CD3^+$ lymphocytes: 4 mL of fasting venous blood was extracted, and mononuclear cells were isolated to detect the levels of $CD3^+CD8^+$ lymphocytes and $CD3^+$ lymphocytes (kit was provided by the BD Company, USA), following in strict accordance with the instructions.

Observation indicators

(1) Treatment effect was judged and classified according to the efficacy. Uncured: the joint movement, swelling, or pain did not change significantly. Improved: the joint pain or swelling and the joint motion function were relieved. Cured: the joint pain or swelling completely disappeared, and the joint motion function returned to normal. The total effective rate = (improved + cured) number of cases/total number of cases × 100% [6]. (2) Serum miR-214 expression level was determined before and after treatment. The higher the level was, the more severe the osteoarthritis was. (3) Inflammatory factors including serum IL-1β, TNF-α, and hs-CRP levels were detected by ELISA before and after treatment. The higher the levels were, the more serious the inflammatory response was. (4) Immune function: the levels of $CD3^+CD8^+$ lymphocytes and $CD3^+$ lymphocytes before and after treatment were detected. The higher the level was, the better the immune function was. (5) Knee function: the Lysholm knee function score was used for evaluation before and at 1 month, 3 months, and 6 months after treatment, with a full score of 100 points. The score was directly proportional to the knee function [7]. (6) 6 months after treatment, the incidence of complications (deformities, deep infections, and severe pain) between the two groups was compared.

Statistical methods

SPSS 20.0 software was applied in this study. Quantitative data were represented by $x \pm s$; the comparison between the two groups was performed by t test, and the comparison of data at different time points between the groups was performed by repeated measurement analysis of variance. Qualitative data were expressed as n (%); the comparison was performed using χ^2 test, and the chi-square value needed to be corrected when 1≤ theoretical frequency <5. A value of $P<0.05$ was considered significant. GraphPad Prism 8 software was used to plot graphics.

Results

Higher treatment effect in the observation group

The observation group had higher total effective rate (93.75%) in comparison to the control group (72.92%) (P<0.05, **Table 2**).

Lower serum miR-214 expression level in the observation group

The serum miR-214 expression level of the two groups was basically the same before treatment (P>0.05). After treatment, the serum miR-214 expression level of the two groups decreased significantly, with a more marked decrease in the observation group (all P<0.05, **Figure 1**).

Lower levels of related inflammatory factors in the observation group

The levels of IL-1β, TNF-α, and hs-CRP were not statistically different in the two groups before treatment (P>0.05). After treatment, IL-1β, TNF-

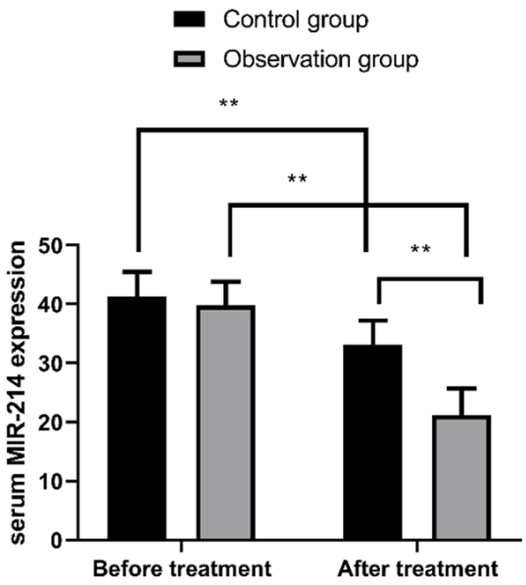

Figure 1. Comparison of serum miR-214 expression before and after treatment between two groups (x ± s). Note: In the control group, the serum miR-214 expression before treatment was (41.35±4.12), while after treatment it was (33.15±4.08); In the observation group, the serum miR-214 expression before treatmentwas (39.76±4.07), while after treatment it was (21.17±4.55). After treatment, the comparison of serum miR-214 expression between the two groups was statistically significant (t = 13.580, **P<0.01). After treatment, serum miR-214 expression in the observation group was lower than that before treatment (t = 11.670, **P<0.01). After treatment, serum miR-214 expression in the control group was lower than that before treatment (t = 10.630, **P<0.01).

α, and hs-CRP in the two groups decreased, and the decrease in the observation group was greater (all P<0.05). See **Table 3**.

Better immune function in the observation group

The $CD3^+CD8^+$ and $CD3^+$ levels were not statistically different between the two groups before treatment (P>0.05). After treatment, the levels of $CD3^+CD8^+$ and $CD3^+$ in the two groups increased, and the increase in observation group was greater (P<0.05). See **Table 4**.

Better knee function in the observation group

The Lysholm score was higher in the observation group than it was in the control group (inter-group effect: F = 58.070, P<0.001), and the Lysholm score of both groups tended to increase with time (time effect: F = 145.900, P<0.001). Grouping and time showed an interactive effect (interactive effect: F = 8.646, P<0.001). See **Figure 2**.

Fewer complications in the observation group

The complication rate of patients in the observation group was lower than that in the control group during 6 months after treatment (P<0.05, **Table 5**).

Discussion

Knee osteoarthritis not only is a degenerative disease of articular cartilage, but also is capable of reducing the synthesis of proteoglycan by chondrocytes under the action of inflammation, resulting in immune function impairment, and further leading to the destruction of articular cartilage and aggravation of inflammation [8]. Knee osteoarthritis is mainly caused by degeneration, strain, infection, or trauma and other factors. Patients often show symptoms of swelling, pain, and limited motion of the knee joint. As a result, their normal life is negatively affected, and cellular inflammatory factors play a key role in the progress of knee osteoarthritis [1]. The present study found that compared to the control group, the total effective rate in the observation group was notably higher; the expression level of serum miR-214 in the observation group was significantly lower; IL-1β, TNF-α, and hs-CRP were lower in the observation group. The $CD3^+CD8^+$ and $CD3^+$ levels of the observation group were significantly higher, and the Lysholm score was higher in the observation group. Given the aforementioned results, it is assumed that the use of Etoricoxib in the treatment of knee osteoarthritis can improve the overall efficacy, reduce the serum miR-214 expression level and inflammatory factor levels, and effectively improve the immune function and knee function compared to the non-steroidal anti-inflammatory drug Celecoxib. The complication rate of patients in the observation group was significantly lower than that in the control group during 6 months after treatment. This is presumably due to the following. (1) Nonsteroidal anti-inflammatory drugs can effectively reduce the activity of COX, inhibit arachidonic acid, and then exert anti-inflammatory and analgesic effects. COX includes two isozymes, COX-1 and COX-2. COX-1 can protect the stomach, regulate platelet aggregation, and periph-

Table 3. Comparison of inflammatory factors before and after treatment between two groups ($\bar{x} \pm s$)

Group	IL-1β (ng/L)		TNF-α (pg/mL)		hs-CRP (μg/mL)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group (n = 48)	169.79±23.16	110.48±22.03[b]	79.13±26.18	54.33±19.15[b]	11.68±2.37	10.22±1.28[b]
Observation group (n = 48)	166.55±23.08	89.14±22.14[b]	79.22±25.89	37.85±19.07[b]	12.08±2.36	9.17±1.16[b]
t	0.687	4.734	0.017	4.225	0.829	4.211
P	0.494	<0.001	0.987	<0.001	0.409	<0.001

Note: b represents P<0.05.

Table 4. Comparison of immune function before and after treatment between two groups ($\bar{x} \pm s$)

Group	$CD3^+CD8^+$ (number·mL^{-1})		$CD3^+$ (number·mL^{-1})	
	Before treatment	After treatment	Before treatment	After treatment
Control group (n = 48)	156.33±60.48	217.82±72.14[c]	581.11±101.02	637.34±100.06[c]
Observation group (n = 48)	154.17±60.52	277.12±65.47[c]	587.14±101.05	850.13±100.08[c]
t	0.175	4.217	0.070	10.420
P	0.862	<0.001	0.944	<0.001

Note: c represents P<0.05.

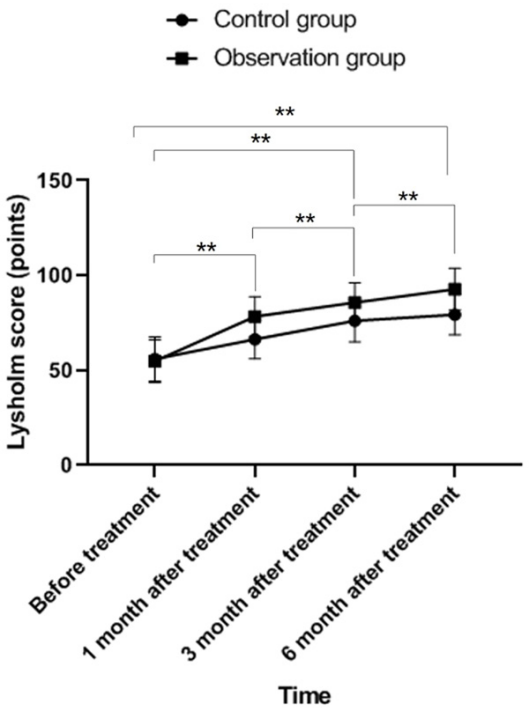

Figure 2. Comparison of Lysholm score between two groups. Note: ** represents P<0.01.

eral vascular resistance, and thus maintain renal blood flow, while COX-2 participates in the synthesis of prostaglandins [9, 10]. Etoricoxib is a COX-2 inhibitor in non-steroidal anti-inflammatory drugs. By inhibiting the synthesis of cyclooxygenase and prostaglandin, it exerts an impressive swelling diminishing and long-lasting analgesic effect on knee osteoarthritis patients, and in turn effectively improves clinical symptoms [11, 12]. (2) microRNAs are a large family of endogenous, non-coding nucleotide sequences, that contain 21 to 25 nucleotides in length, and mainly regulate gene expression through the function of post-transcription messenger RNA [13]. Studies have proved that miRNAs are involved in the occurrence and development of orthopedic diseases, and miR-214 plays a part in regulating osteoclast function [14, 15]. It has been reported in studies that miR-214 may be involved in the process of cartilage and subchondral bone damage in the progress of knee osteoarthritis, promoting the occurrence and development of osteoarthritis [16, 17]. High expression levels of miR-214 can further regulate cartilage degradation and exacerbate knee osteoarthritis cartilage damage. Etoricoxib can notably reduce the expression level of miR-214, thereby reducing the damage of knee osteoarthritis cartilage and delaying the progression of the disease. (3) Etoricoxib inhibits the synthesis of prostaglandins, significantly reducing the level of serum inflammatory factors in patients, and further relieving the inflammatory response and playing an anti-inflammatory effect [18]. (4) Etoricoxib can inhibit some of the enzymes involved in the destruction of cartilage. On the other

Table 5. Comparison of the incidence of complications between the two groups

Groups	deformities	deep infections	severe pain	incidence rate
Control group (n = 48)	3	1	3	14.58%
Observation group (n = 48)	0	0	1	2.08%
X^2				4.909
P				0.027

hand, it can significantly reduce the release of endotoxic factors in inflammatory transmitters and damaged cells, which helps to improve the body's immune function [19]. (5) Etoricoxib promotes knee joint function by improving the overall curative effect of knee osteoarthritis [20]. Etoricoxib is a new generation of highly selective COX-2 inhibitors, which not only have strong antipyretic, analgesic, and anti-inflammatory effects, but also slightly affect gastrointestinal tract and platelet function, and are well tolerated by patients [12]. However, due to the small sample size, there may be a certain bias in the results, and studies with larger sample sizes are needed.

In conclusion, Etoricoxib has a satisfactory therapeutic effect on patients with knee osteoarthritis, and can notably reduce the expression of serum miR-214 and the level of inflammatory factors, which is worthy of clinical application.

Disclosure of conflict of interest

None.

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