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Comparison of Therapeutic Effects of Topical Application of Diclofenac Sodium Nanoparticles and Conventional Placebo on Knee Osteoarthritis

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ABSTRACT

The objective of this study was to investigate the therapeutic effects of diclofenac sodium nanoparticles and traditional placebo on Knee Osteoarthritis (KOA). 80 KOA patients admitted to the Inpatient Department of Orthopedics of Sunshine Union Hospital from June 2020 to July 2021 were selected, and divided into 40 cases using diclofenac sodium nano flexible liposomes topically (study group) and 40 cases using conventional placebo topically (control group). The two groups were compared for knee swelling, knee pain, and knee function scores before treatment, on the 3rd, 7th, and 14th days after treatment, and the incidence of adverse events during treatment was recorded. It was found that the knee swelling scores of the study group were significantly lower than those of the control group on the 7th and 14th days after treatment, and that the knee pain score of the study group was significantly lower than that of the control group at the 7th day after treatment, and that the knee joint mobility disorder scores of the study group were significantly lower than those of the control group at the 7th and 14th days after treatment. The total effective rate of the study group was 85.0% (34/30), which was higher than that of the control group (12.5% (5/30)) (P<0.05). In conclusion, local application of diclofenac sodium nano flexible liposomes showed a stronger effect on KOA than traditional placebo, and no significant adverse reactions occurred.

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Introduction

Osteoarthritis (OA) is a common and frequently-occurring disease in orthopedics, also known as degenerative arthropathy, hyper osteogeny, and osteoarthropathy, characterized by degeneration and loss of articular cartilage, subchondral bone sclerosis or cystic change, and marginal hyper osteogeny (1). OA is common in people over 50 years old, and it mostly involves the weight-bearing joints such as the hip, knee, and spine. Studies have shown that westerners are predominantly affected by OA of the hip joint, while easterners mainly suffer from knee osteoarthritis (KOA) (2). KOA is the most common OA. It is a chronic low inflammatory joint degenerative disease affecting the whole joint. It mainly features degenerative changes in knee cartilage as well as osteophytes, and sclerosis (3,4). In China, KOA is the most common joint disease in middle-aged and elderly people. According to statistics, OA is one of the main causes of lower limb dysfunction in the elderly population. The survey shows that about 40% of men and 47% of women are affected by OA (5). Under the combined stimulation of many factors, the abnormal coupling of cartilage matrix decomposition and synthesis leads to cartilage and subchondral bone damage, which then affects the surrounding tissue, followed by pathological changes such as synovitis, narrowing of joint space, and spur formation. Clinically, it manifests as knee joint pain, stiffness, limited mobility, joint deformity in the late stage, and even disability, leading to complete loss of knee joint function (6). KOA is common in middle-aged and elderly people, and the incidence of KOA is different between men and women. The incidence of KOA in women is higher than that in men, especially in postmenopausal women. In China, the prevalence of symptomatic KOA has reached 8.1%. With the population aging, its prevalence continues to rise.

The treatment of KOA aims at relieving pain, restoring joint motion function, and improving patients' quality of life (7). Non-drug therapy is given priority for the treatment of KOA in the early and middle stages, but when the treatment is ineffective, it is necessary to cooperate with drugs to strengthen the

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curative effect. Surgical treatment is suitable for patients with advanced KOA with severe joint pain or joint deformity. Non-steroidal anti-inflammatory drugs are the most commonly used drugs, such as diclofenac sodium. Its main mechanism of action is to inhibit cyclooxygenase to prevent the metabolism of arachidonic acid, thereby reducing the synthesis of prostaglandins and the production of platelets, and to inhibit lipoxygenase to a certain extent to reduce the production of leukotriene, bradykinin, and other products, especially leukotriene B4. By inhibiting these inflammatory factors, it plays an antipyretic, analgesic, and anti-inflammatory role. The mechanism of diclofenac sodium in treating arthritis pain (8) is to reduce the production of prostaglandins by inhibiting the activity of cyclooxygenase which is highly expressed in injured nerve and spinal cord, so that the expressions of substance P (SP) and calcitonin gene-related peptide (CGRP) in the superficial layer of dorsal root ganglion and spinal cord can be correspondingly reduced, thus reducing the pain. However, if the drug is taken orally for a long time, it will increase the risk of digestive, cardiovascular, blood and other systemic diseases, and the efficacy will be greatly weakened due to the first-pass effect (9). To reduce the side effects caused by long-term oral administration, topical local administration is usually taken. Of the topical preparations, diclofenac sodium emulsion is frequently used. Diclofenac sodium emulsion is also called Fortalin, but because of its good solubility, the skin penetration rate of Fortalin emulsion is low, only about 6%.

In recent years, the research on the technology to promote drug transdermal absorption has focused on mechanical methods, including ion ultrasonic introduction, electric breakdown, heat energy, microneedle, laser, and other technologies. Nevertheless, they have limited improvement of transdermal efficiency and are traumatic. Consequently, they are rarely used clinically. As a new drug carrier, nano-flexible liposome is to add surfactants such as sodium cholate to the phospholipid bilayer of the ordinary liposome. It has many excellent characteristics, such as being suitable for in vivo degradation, non-toxic, and non-immunogenic, being able to encapsulate water-soluble and fat-soluble drugs, protecting encapsulated drugs, reducing the dosage of drugs, and being able to modify to produce active targeting (10,11). Meanwhile, the structural characteristics of the liposome lipid bilayer determine its good permeability to the lipid barrier of the skin stratum corneum. However, due to the similar characteristics of liposomes and skin lipids, the retention of liposomes in the skin increases and the amount of the emulsion entering the systemic circulation decreases. In this study, diclofenac sodium nano-flexible liposomes and traditional placebo were compared for the therapeutic effects on KOA patients, expected to enhance the bioavailability of diclofenac sodium and provide a reference for clinical development of new preparations for KOA.

Materials and methods

Research subjects

A total of 80 KOA patients admitted to the Inpatient Department of Orthopedics of Sunshine Union Hospital from June 2020 to July 2021 were selected as research subjects, including 42 females and 38 males, aged from 40 to 69 years old and averaged 54±3.17 years old. They were randomly divided into two groups: the study group (n=40) and the control group (n=40). The patients in the study group were given diclofenac sodium nano-liposomes for external use, and those in the control group were given paraffin wax as a placebo.

Inclusion criteria: (i) diagnosed with KOA according to *the Guidelines for the Diagnosis and Treatment of Osteoarthritis* released by the Joint Surgery, Chinese Society of Osteology in 2018; (ii) with no contraindications for contrast agents; (iii) older than 40 years old and younger than 69 years old; and (iv) not taking drugs orally or external treatment for KOA within 1 month before the experiment.

Exclusion criteria: (i) combined with fracture lesions of the knee joint; (ii) complicated with other infectious diseases or more serious rheumatoid arthritis; (iii) accompanied by serious cardiovascular and cerebrovascular diseases, or liver and kidney dysfunction; and (iv) those with mental and consciousness disorders.

All patients and their families had signed informed consent, and the study had been approved by the Medical Ethics Committee of Sunshine Union Hospital.

Preparation of diclofenac sodium flexible liposomes

In this study, the preparation of diclofenac sodium flexible liposome referred to the method of published literature (12), and it was slightly modified. With 350mg fabaceous lecithin, 50mg sodium cholate, and 50mg diclofenac sodium as the main matrix, a semi-solid colloidal substance was obtained by water bath ultrasonic method, and then passed through 0.22μm microporous membrane after vacuum drying. Finally, diclofenac sodium nano-flexible liposomes were obtained using glycerin and other surfactants.

Treatment methods

40 patients in the study group were given diclofenac sodium nano flexible liposomes for external use, and 40 patients in the control group were given paraffin wax as a placebo. The dosage of the two groups was between 0.5-1g according to the size of the knee joint, 3 times a day, directly applied to the skin surface of the knee joint. Subsequently, palm massage for 3min can promote absorption.

Observation indicators

Observation time was calculated from the first day of admission, namely, from before treatment, to the third, seventh, and fourteenth days after treatment. The observation indicators include three parts: (A) changes in knee function before and after treatment: i. knee swelling score: no swelling at the knee is denoted 0, slight redness is denoted 1, red skin with slight swelling is denoted 2, and obvious redness and fever are denoted 3; ii. knee pain score: 0 points for no pain, 1 point for slight pain, 2 points for obvious pain, and 3 points for very strong pain; iii. knee motion disorder score: 0 points for free movement, 1 point for slight limitation of activity, 2 points for obvious limitation of activity, but the patient can move by himself, and 3 points for movement disorder, and the patient has to stay bed-rest; (B) the treatment effect of patients in the two groups: the improvement degree of clinical symptoms and signs more than 70% is considered remarkably effective; the improvement degree of clinical symptoms and signs greater than 50% and less than 70% is considered effective, the improvement degree greater than 20% and less than 50% is considered slightly effective, and the improvement degree less than 20% is considered ineffective. Total effective rate = (remarkably effective + effective) ×100%; (C) adverse events during treatment: adverse events in this study include itching and rash at the application site, nausea, and dizziness.

Statistical methods

SPSS 20.0 statistical software is used to analyze all data in this study. The Chi-square test is employed to analyze the difference between groups. $P<0.05$ indicates that the difference was statistically significant.

Results and discussion

General data for all patients

Table 1 showed the general clinical data of all patients. There were no significant differences in gender, age, body mass index (BMI), and medical history between the two groups ($P>0.05$), indicating comparability.

Table 1. General data for all patients

Group	Gender (male/female)	Age	BMI(kg/m^2)	Medical history (years)
Research group	18/22	53±3.1	23.80±2.41	0.9±0.13
Control group	20/20	52±3.9	23.73±2.66	0.9±0.15
P	0.179	0.794	0.835	0.820

Changes in knee function in the two groups before and after treatment

Figure 1 shows the changes in knee swelling scores in the two groups before and after treatment. Knee swelling scores in both groups showed a downward trend after treatment, and the decrease degree was more obvious at 7d and 14d after treatment, with statistically significant differences ($P<0.05$). The knee swelling scores of the study group were 1.58±0.61 and 0.94±0.59 at 7d and 14d after treatment, respectively, which were significantly lower than 2.10±0.62 and 1.93±0.58 in the control group at the same period, and the differences were statistically significant. There was no significant improvement in knee swelling in KOA patients in the two groups within 3 days after treatment, but it was significantly improved on the 7th day after treatment, indicating that both treatments were effective. The knee swelling score of the study group was significantly lower than that of the control group on the 7th and 14th days after treatment, indicating that the effect of the study group on knee

swelling was better than the control group on the 7th and 14th days after treatment.

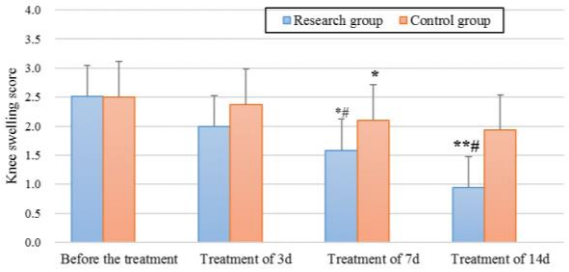

Figure 1. Changes in knee joint swelling between the two groups before and after treatment. *P<0.05, **P<0.01 indicate statistically significant differences compared with before treatment; #P<0.05 indicates statistically significant differences compared with the control group during the same period

Figure 2 shows the changes in knee pain scores in the two groups before and after treatment. It was noted that knee pain scores in both groups showed a downward trend after treatment, and the decline was more obvious on the 7th day after treatment, with statistically significant differences (P<0.05). The knee pain score of the study group was 1.28±0.47 on the 7th day after treatment, which was significantly lower than that of the control group (2.43±0.58) during the same period, with a statistically significant difference. There was no significant improvement of knee pain in KOA patients in the two groups within 3 days after treatment, but it was significantly improved on the 7th day after treatment, indicating that both treatments were effective in relieving knee pain. On the 7th day after treatment, the knee pain score of the study group was significantly lower than that of the control group, indicating that the effect of the study group on knee pain was significantly better than that of the control group on the 7th day after treatment.

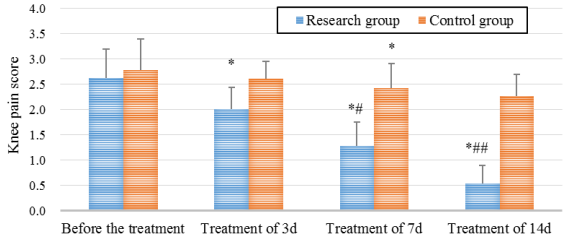

Figure 2. Knee pain scores between the two groups before and after treatment. *P<0.05 indicates statistically significant differences compared with before treatment; #P<0.05, ##P<0.01 indicates the comparison with the control group at the same period is statistically significant

Figure 3 shows the changes in knee joint mobility disorder scores in the two groups before and after treatment. It was noted that knee joint mobility disorder scores in both groups showed a downward trend after treatment, and the decline was more obvious on the 7th and 14th day after treatment, with statistically significant differences (P<0.05). The scores of knee joint mobility disorder in the study group were 1.24±0.45 and 1.05±0.42 at 7d and 14d after treatment, respectively, which were significantly lower than 1.97±0.58 and 1.82±0.51 in the control group at the same period, and the differences were statistically significant. There was no significant improvement of knee joint mobility disorder in KOA patients in the two groups within 3 days after treatment, but it was significantly improved on the 7th and 14th days after treatment, indicating that both treatment methods were effective in alleviating the knee joint mobility disorder in KOA patients. Especially at 7 and 14 days after treatment, the scores of knee joint mobility disorder in the study group were significantly lower than those in the control group, indicating that the effect of knee joint mobility disorder in the study group was better than that in the control group.

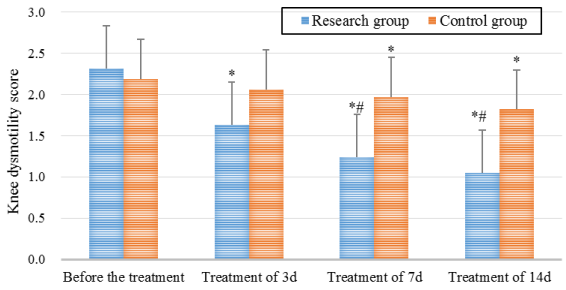

Figure 3. Comparison of knee joint mobility disorder scores between the two groups before and after treatment. Note: *P<0.05 indicates statistically significant differences compared with the group before treatment; #P<0.05 indicates statistically significant differences compared with the control group during the same period

Comparison of treatment effects between the two groups

Table 2 and Figure 4 show the treatment results of all patients. The total effective rate at 14 days of treatment was 85.0% (34/30) in the study group and 12.5% (5/30) in the control group. Compared with the control group, the treatment efficiency of patients in the study group was higher, indicating that diclofenac sodium nano flexible liposomes had a better

therapeutic effect on KOA patients than traditional placebo, with a statistically significant difference (P<0.05).

Table 2. Effect comparison between the two groups after 14 days of treatment

Group	Markedly effective	Effective	Minimal effect	Invalid
Research group	20	14	6	0
Control group	0	5	5	30

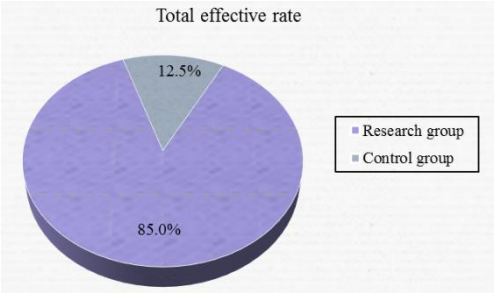

Figure 4. The total effective rates in the two groups.

Incidence of adverse events in the two groups

Table 3 and Figure 5 show adverse events in the two groups. 1 patient in the study group experienced pruritus at the site of drug application and 1 patient in the control group experienced rash during treatment. The overall incidence of adverse events in the study group was 5.0% (2/40), higher than that in the control group (2.5% (1/40)). However, there was no significant difference between the two groups (P>0.05), indicating that the two treatment methods had no obvious skin irritation and other side effects for KOA patients.

Table 3. Incidence of adverse events between the two groups during treatment

Group	Itching	The rash	Nausea	Dizzy
Research group	1	1	0	0
Control group	0	1	0	0

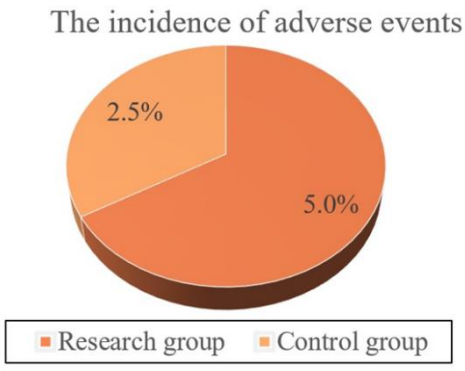

Figure 5. The total incidence of adverse events during treatment in the two groups.

KOA is a progressive and degenerative joint disease that spreads to the trunk and limbs. The degeneration of knee cartilage destroys the integrity of the joint, and the degeneration of the force line and joint itself leads to subchondral bone lesions. Thus, patients manifest a series of clinical syndrome (13). As a common chronic joint disease in orthopedics, KOA is mainly characterized by degenerative changes of articular cartilage and secondary hyper osteogeny, which can cause knee pain, stiffness, and dysfunction (14). The risk factors of KOA mainly include age, obesity, and gender, which seriously affect the quality of life of patients and easily cause depression and anxiety (15). The prevalence rate of KOA among adults in Portugal is about 12.4% (16), and the total prevalence rate of KOA among adults in China is about 15%. The prevalence rate of KOA among people over 40 years old is 10%~17%, that of people over 60 years old is 50%, and that of people over 75 years old is as high as 80% (17). The prevalence rate of KOA is not only increased with age but is also closely related to gender. For example, the prevalence rate of KOA among men over 40 years old in China gradually increased from 4.3% to 27.3%, while that of women gradually increased from 8.8% to 42.7% (18).

The clinical pathogenesis of KOA is still unclear. The stimulation of articular chondrocytes and synovial cells will lead to inflammatory reaction and injury and the production of many cytokines, mainly interleukin-8 (IL-8), interleukin-1 (IL-1), which will induce chemotaxis of granulocytes and monocytes macrophages and will release oxygen free radicals, lysosomal enzyme, and histamine, so as to degrade cartilage matrix. Meanwhile, it can also induce the activation of B cells and T cells, decrease immunity, and aggravate body injury (19). The etiology of KOA has not been determined, so there is no clear treatment. Drug therapy is a common clinical method, such as injecting glucocorticoid drugs into joints. Hormones can effectively inhibit the degradation of joint fluid in KOA patients, which may be related to the inhibition of MMPs activity, greatly improving the symptoms. However, the massive use of hormones can lead to cartilage repair disorder and secondary damage to bone joints (20). Commonly used drugs are nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac sodium, which is suitable for patients with moderate to severe arthritis and has good

analgesic effects. This medicine can be divided into the tendentious cyclooxygenase-2 inhibitor, non-tendentious cyclooxygenase inhibitor, and specific cyclooxygenase-2 inhibitor. Non-tendentious cyclooxygenase inhibitor can effectively inhibit cyclooxygenase-2 and cyclooxygenase-1 and has analgesic and anti-inflammatory effects, but it has certain toxicity and gastrointestinal adverse reactions to the kidney, so elderly patients should be careful to use it. The tendentious cyclooxygenase-2 inhibitor has stronger effects on cyclooxygenase-2 than cyclooxygenase-1 and has higher safety. Specific cyclooxygenase-2 inhibitors have a strong effect on cyclooxygenase-2, but have no effect on cyclooxygenase-1, and have nephrotoxicity. Moreover, safety, when used in the cardiovascular system, needs in-depth clinical observation (21). Long-term oral administration of diclofenac sodium is easy to destroys the gastric mucosal barrier and cause gastric ulcer, which often leads to digestive tract reactions such as nausea, vomiting, and upper abdominal fullness. When this drug is made into external preparations, such as flexible nano-liposomes, as a new type of transdermal drug delivery carrier, with high self-deformation, it can efficiently pass through skin pores several times smaller than itself and enable some macromolecular drugs that are difficult to penetrate skin successfully penetrate or even enter systemic circulation, eliminating the first-pass effect and exerting greater effects (22-27).

In this experiment, diclofenac flexible nano-liposomes were applied topically to KOA patients. The results showed that the knee joint swelling scores at 7 days and 14 days after topical diclofenac flexible nano-liposomes treatment (1.58±0.61,0.94±0.59) were significantly lower than those of the control group (2.10±0.62,1.93±0.58). The knee joint pain score of the study group on the 7th day after treatment (1.28±0.47) was significantly lower than that of the control group (2.43±0.58). On the 7th and 14th days after treatment, the scores of knee joint mobility dysfunction in the study group (1.24±0.45, 1.05±0.42) were significantly lower than those in the control group (1.97±0.58, 1.82±0.51), and the differences were statistically significant (P<0.05). On the 14th day after treatment, the total effective rate of the study group was 85.0%(34/30), which was significantly higher than that of the control group (12.5%(5/30) (P<0.05). The total incidence of adverse events in the study group was 5.0%(2/40), which was higher than that in the control group (2.5%(1/40)), but there was no significant difference between the two groups (P>0.05).

Conclusions

In this study, diclofenac flexible nano-liposomes were used for topical treatment of KOA compared with a traditional placebo. The results showed that diclofenac flexible nano-liposomes could improve the knee function of patients with KOA by alleviating knee swelling, knee pain, and knee joint mobility disorder. Compared with a traditional placebo, the total effective rate of diclofenac flexible nano-liposomes (85.0%) was higher than that of the control group (12.5%), and no significant adverse reactions occurred compared with the control group. However, some limitations in the study should be noted. The sample size is small, which will reduce the power of the study. In the follow-up, expanded sample size is necessary to strengthen the findings of the study. In conclusion, this study provides a reference for the development of diclofenac flexible nano-liposomes as an ideal topical drug for the treatment of KOA.

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

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