

Short Research Article

The effects of ozone therapy on the expression of IL-8, IL-10, CXCL9 and CXCL11 in joint fluid in patients with knee osteoarthritis

Abstract

Knee joint is one of the common sites of osteoarthritis (OA) with manifestation of localized joint swelling, pain and deformity, often seriously affecting daily work and life. It was found that the activation of Janus kinase / signal transducer and activator of transcription 3 (JAK / STAT3) signal pathway can lead to osteoarthritis. JAK-STAT signal transduction pathway is closely related to painful diabetic peripheral neuropathy and participates in neuropathic pain. Ozone therapy is often used for alleviating pain in neck, shoulder, waist and leg due to its oxidative, anti-inflammatory and analgesic effects. In recent years, ozone therapy is promising in treating knee osteoarthritis, which can significantly reduce the pain and swelling of the joints [1,2]. The mechanism of ozone for knee osteoarthritis remains unclear and might associated with inhibition of inflammatory factors and matrix metalloproteinase activity, and intervention of free radical metabolism [3]. In this study, the efficacy of ozone in the treatment of OA and its effect on inflammatory factors were evaluated by knee joint cavity ozone injection and detecting the expression of inflammatory factors IL-8, IL-10, CXCL9 and CXCL11 in joint fluid.

Keywords Knee joint; Osteoarthritis; Ozone; Pain; Inflammatory mediators

Introduction

Osteoarthritis (OA) is a kind of joint degenerative disease, which is characterized by local joint swelling, pain and deformity. It often occurs in the weight-bearing joints of lower limbs, especially the knee joint, and often seriously affects daily work and life. The main pathological changes were apoptosis and destruction of bone cells, synovitis, and then subchondral bone hyperplasia. **Knee osteoarthritis is a common disease in the elderly.** The World Health Organization reported that the prevalence of OA in the elderly aged 65 ~ 70 is close to 62%, and nearly 70% in the population over 70. **With the advent of China's aging society, the treatment of OA will become an urgent problem in geriatrics in China.**

Medical trioxide has strong oxidation ability and anti-inflammatory and analgesic effects. It is mainly used for anti-inflammatory and analgesic treatment of neck, shoulder, waist and leg pain. In recent years, medical trioxide has achieved good clinical results in the treatment of knee osteoarthritis, which can significantly reduce the pain and swelling of patients' joints. The exact target of medical trioxide in the treatment of knee osteoarthritis is not clear, which may be related to the inhibition of inflammatory factors, matrix metalloproteinase activity and intervention of free radical metabolism.

1 Methods

1.1 Study population

According to the diagnostic criteria for knee OA in the “Osteoarthritis Diagnosis and Treatment Guidelines (2018 edition)” [4], 20 patients with advanced knee osteoarthritis participated our study from January 2019 to March 2020 at Second People's Hospital of Foshan. All patients received no drugs or other treatments within 2

weeks before outpatient visiting. There was neither skin broken or infection around the knee joint, nor do patients have previous cardiopulmonary disease, obvious impairment of liver and kidney function, history of infectious diseases and autoimmune diseases, or history of drug allergies. Patients with ISOA (index of severity for knee joint osteoarthritis) score over 14 were excluded.

1.2 Interventions

The patients were placed in spine position with both legs straightened. Expose the affected knee joint, disinfecting and draping as usual. Local anesthesia was performed with 1% lidocaine hydrochloride 2mL, then puncture the joint cavity under ultrasound guide. Firstly, 4mL of effusion was extracted and storage in -70°C refrigerator. After that, a 20mL gas mixture of air and ozone were injected into the joint cavity. Ozone was prepared by ozone therapy device (Ozomed® Smartline, Kastner-Praxisbedar, German) with concentration of $30\ \mu\text{g}/\text{mL}$. Finally, puncture point was bandaged by sterile patch. The above procedure was repeated after 7d for each patient.

1.3 Outcomes

Visual simulation scoring (VAS) was performed at the following time points: before initial ozone injection (T0), 3d after initial ozone injection (T1), 7d after initial ozone injection (T2), 7d after therapy finished (T3) and 14d after therapy finished (T4). Efficacy was assessed at T0 and T4 according to Michel Lequesne index of severity for knee joint osteoarthritis (ISOA), which range from 0 to 24 and the higher score means more severe condition (mild <5 , moderate 5-7, severe 8-10, very severe 11-13, extremely severe >14). The expression of IL-8, IL-10, CXCL9 and CXCL11 in joint fluid was evaluated via enzyme-linked immunosorbent assay (ELISA) according to the kits (R&D System, USA) manual.

1.4 Statistical analysis

All data were expressed as mean±SD and analyzed using SPSS version 17.0 (SPSS Inc.). VAS scores at all time points were compared using one-way ANOVA. ISOA scores and inflammatory factor expressions before and after therapy were analyzed by paired *t*-test. $P<0.05$ was considered to indicate a statistically significant difference.

2 Results

2.1 Study population

20 patients with advanced knee osteoarthritis were finally involved. There were 6 males and 14 females at the age of 65 ± 8 , whose course of disease were 41 ± 5 months.

All patients received intraarticular ozone injection and no complications such as infections or hemorrhage occurred.

2.2 VAS and ISOA scores

All patients suffered mild to severe pain before therapy whose VAS score was 8.3 ± 1.0 . After initial ozone therapy, VAS score decreased to 5.5 ± 1.0 . The score of 7d and 14d after therapy finished is 2.1 ± 1.3 and 2.3 ± 1.2 , the difference being statistically significant ($P<0.05$) compared with before-therapy VAS score, as is shown in table 1. Mild to severe joint dysfunction exists in all patients before therapy with ISOA score of 8.6 ± 1.3 . The score decreased to 4.0 ± 1.2 at 14d after therapy finished and compared with before-therapy score, the difference is statistically significant ($P<0.05$), as is shown in table 1.

Table 1: VAS and ISOA score (n=20, Mean±SD)

	T0	T1	T2	T3	T4
VAS	8.3±1.0	5.5±1.0 ^a	3.5±1.1 ^{ab}	2.1±1.3 ^{abc}	2.3±1.2 ^{abc}
ISOA	8.6±1.3				4.0±1.2 ^a

Compared with T0, ^a*P*<0.05; compared with T1, ^b*P*<0.05; compared with T2, ^c*P*<0.05

2.3 The expression of IL-8, IL-10, CXCL9 and CXCL11 in joint fluid

Before ozone therapy (T0), the concentration of IL-8, IL-10, CXCL9 and CXCL11 was 40±6 pg/mL, 5.4±1.0 pg/mL, 990±162 pg/mL and 420±68 pg/mL. 7d after initial therapy (T2), the concentration of these inflammatory factors mentioned above was 27±4 pg/mL, 9.5±1.6 pg/mL, 642±93 pg/mL and 222±57 pg/mL. The concentration of IL-8, CXCL9 and CXCL11 were significantly decreased (*P*<0.05) while IL-10 was significantly increased (*P*<0.05) compared with that of before-therapy, as is shown in table 2.

Table 2: Expression of IL-8, IL-10, CXCL9 and CXCL11 (n=20, Mean±SD, pg/mL)

	IL-8	IL-10	CXCL9	CXCL11
T0	40±6	5.4±1.0 ^a	990±162	420±68 ^{abc}
T2	27±4 ^a	9.5±1.6 ^a	642±93 ^a	222±57 ^a

Compared with T0, ^a*P*<0.05

3 Discussion

KOA is a common disease in geriatric patients with mainly pathological changes

of apoptosis and destruction of bone cells, synovitis, and subsequent subchondral osteoporosis. Treatment of OA will become urgent as the coming of China's aging society. OA is an inflammatory disease induced by various of inflammatory mediators [6-8]. Laboratory tests showed that the serum C-reactive protein, IL-6, IL-8, CXCL9, and CXCL11 levels in OA patients were higher than normal, suggesting that inflammatory response was involved in the pathogenesis of OA. Synovitis can occur in both early and advanced stage of knee osteoarthritis and is associated with joint pain, degeneration, and defects. Inflammatory cytokines such as IL-6, TNF- α , IL-1 and JAK-STAT are all associated with the occurrence of OA. TNF- α plays an important role in matrix degradation and bone destruction in osteoarthritis, and it induces the production of other cytokines such as IL-6 and matrix metalloproteinase. Studies have shown that IL-1 β is associated with pain and the severity of radiological manifestations of joint structures in OA patients. Intraarticular injection of IL-1 β receptor antagonists can improve synovial inflammation and hyperplasia, reduce the level of high-sensitivity C-reactive protein, and facilitate the treatment of OA.

As a strong oxidant, ozone can eliminate free radicals in the joints of KOA patients and improve the environment of joint cavity, thus it is often used for the treating chronic pain. Studies have shown that ozone could reduce the expression of inflammatory factors in the joint fluid of KOA patients and decrease inflammatory responses [1,9]. Our study found that intra-articular injection of ozone could relieve the pain of KOA patients, improve joint function, and reduce VAS and ISOA scores, suggesting that intra-articular injection of ozone is effective in relieving pain and improving joint function of KOA patients. ELSA assay of IL-8, IL-10, CXCL9, CXCL11 showed that ozone can reduce the content of inflammatory factors IL-8, CXCL9 and CXCL11 in the joint fluid of KOA patients, increase the content of anti-inflammatory factor IL-10,

suggesting that ozone might alleviate the pain and improve joint function of KOA patients by regulating inflammatory response.

Meanwhile, Attention should be paid for very severe KOA patients as ozone might be ineffective or even deteriorate the conditions due to its strong oxidizing property. Dose and frequency should also be cautious to reduce adverse effects. In summary, intra-articular ozone injection could alleviate the pain of KOA patients and the mechanism may associated with decreasing of inflammatory factors and promoting of anti-inflammatory factors in joint fluid.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

- [1] Sconza C, Respizzi S , Virelli L, et al. Oxygen-Ozone Therapy for the Treatment of Knee Osteoarthritis: A Systematic Review of Randomized Controlled Trials. *Arthroscopy*. 2020;36(1):277-286. doi: 10.1016/j.arthro.2019.05.043.
- [2] Raeissadat SA , Rayegani SM, Forogh B, et al. Intra-articular ozone or hyaluronic acid injection: Which one is superior in patients with knee osteoarthritis? A 6-month randomized clinical trial. *J Pain Res*. 2018 ;11:111-117. doi: 10.2147/JPR.S142755. eCollection 2018.
- [3] Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis[J]. *Clin Orthop Relat Res*, 2004, 427 (1): 27-36.
- [4] Joint surgery group, branch of Orthopaedics, Chinese Medical Association, guidelines for the diagnosis and treatment of osteoarthritis (2018 Edition), *Chinese Journal of Orthopaedics*, 2018, 38 (12): 705-715.
- [5] DawsonJ, LinsellL, Doll H, et al. AssessmentoftheLequesneindexofseverityforosteoarthritisofthehipinanelderlypopulation. *OsteoarthritisCartilage*, 2005, 13(10): 854-860.
- [6] Chow YY, Chin KY .The Role of Inflammation in the Pathogenesis of Osteoarthritis. *Mediators Inflamm*. 2020 Mar 3;2020:8293921. doi:

10.1155/2020/8293921. eCollection 2020.

- [7] Yang P, Tan J, Yuan Z, et al. Expression profile of cytokines and chemokines in osteoarthritis patients: Proinflammatory roles for CXCL8 and CXCL11 to chondrocytes. *Int Immunopharmacol.* 2016;40:16-23. doi: 10.1016/j.intimp.2016.08.005.
- [8] Malemud CJ, Negative Regulators of JAK/STAT Signaling in Rheumatoid Arthritis and Osteoarthritis. *Int J Mol Sci.* 2017;18(3). pii: E484. doi: 10.3390/ijms18030484.
- [9] Oliviero A , Giordano L, Maffulli N. The temporal effect of intra-articular ozone injections on pain in knee osteoarthritis. *Br Med Bull.* 2019;132(1):33-44. doi: 10.1093/bmb/ldz028.

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