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Changes of the cytokine profile in patients with traumatic synovitis on the background of ozone therapy

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Abstract

Aim – the cytokine profile research in patients with posttraumatic synovitis during intravenous and intra-articular ozone therapy.

Material and methods. The research involved 69 patients with traumatic knee joint damage complicated by posttraumatic synovitis. Patients in Group I (35 people) received a traditional treatment. Patients in Group II (34 people) along with the traditional therapy had intravenous injection of 200 ml of ozonated solution of sodium chloride in ozone concentration 2 mg/l once daily for 10 days, and intra-articular injection of 20 ml ozone-oxygen mixture in ozone concentration of 15 mg/l once every second day, in an amount of 5 injections.
During arthroscopy, the lavage of the joint cavity was performed with ozonated saline solution at a concentration of 2.0 mg/l.
The cytokine profile was evaluated by the content of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-17), regulatory (IL-2), IL-1 receptor antagonist, and anti-inflammatory (IL-4, IL-10) cytokines by solid-phase enzyme immunoassay with peroxidase as an indicator. Statistical analysis of the results was carried out using the Student t-test.

Results. The use of intravenous and intra-articular ozone therapy contributed to synchronous decrease of the levels of pro-inflammatory cytokines with simultaneous reduction of anti-inflammatory mediators of inflammation. TNF-α content decreased by 24.6% (p_2<0.001), IL-17 – by 17.3% (p_2<0.01), IL-6 – by 20.1% (p_2<0.001), IL-1β – by 19.1% (p_2<0.001), IL-2 – by 25.7% (p_2<0.001), IL-1RA – by 24.4% (p_2<0.001), IL-10 – by 21.3%(p_2<0.001), IL-4 – by 25.7% (p_2<0.001) in comparison to traditional treatment.

Conclusion. The complex ozone therapy led to the decrease of inflammation, which was reflected in the depression dynamics of the studied cytokines. These results allow us to evaluate it as an effective treatment method for post-traumatic synovitis which effectively reduces the secondary posttraumatic alteration of tissue structures.

Keywords: joint injury, synovitis, inflammation, cytokine profile, interleukins, ozone therapy.

Conflict of interest: nothing to disclose.

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Динамика цитокинового профиля у больных с посттравматическим синовитом на фоне озонотерапии

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Аннотация

Цель – изучить цитокиновый профиль у пациентов с посттравматическим синовитом при внутривенной и внутрисуставной озонотерапии.

Материал и методы. Работа основывается на результатах исследования и лечения 69 больных с травматическими повреждениями коленного сустава, осложненными развитием посттравматического синовита.
Больные I группы (35 человек) получали комплексное традиционное лечение. У пациентов II группы (34 больных) традиционная терапия дополнялась 10-дневным курсом внутривенной инъекционной озонотерапии: 200 мл раствора NaCl в концентрации 2,0 мг/л 1 раз в сутки и внутрисуставным введением 20 мл озона в концентрации 15 мг/л через день в количестве 5 процедур.
Во время выполнения артроскопии производился проточно-промывной лаваж сустава озонированным раствором NaCl в концентрации 2,0 мг/л.
Цитокиновый профиль исследовался по содержанию провос-

палительных (TNF-α, IL-1β, IL-6, IL-17), регуляторного (IL-2), рецепторного антагониста Ил-1 и противовоспалительных (IL-4, IL-10) цитокинов методом твердофазного иммуноферментного анализа с индикаторной меткой в виде пероксидазы. Статистический анализ результатов проведен посредством критерия Стьюдента.

Результаты. Применение внутривенной и внутрисуставной озонотерапии способствовало синхронному снижению уровня провоспалительных цитокинов одновременно с ограничением роста противовоспалительных медиаторов воспаления. Содержание TNF-α уменьшилось на 24,6% (p_2<0,001), IL-17 – на 17,3% (p_2<0,01), IL-6 – на 20,1% (p_2<0,001), IL-1β – на 19,1% (p_2<0,001), IL-2 – на 25,7% (p_2<0,001), IL-1RA – на 24,4% (p_2<0,001), IL-10 – на 21,3%(p_2<0,001), IL-4 – на 25,7% (p_2<0,001) по отношению к данным, полученным при традиционном лечении.

Заключение. Применение в комплексной терапии озонотерапии приводило к снижению воспалительной реакции, что отразилось в динамике депрессии показателей исследуемых цитокинов. Данные результаты позволяют оценивать этот метод в лечении посттравматического синовита как высокоэффективный и позволяющий эффективно уменьшить вторичную посттравматическую альтерацию тканевых структур.

Ключевые слова: травма сустава, синовит, воспаление, цитокиновый профиль, интерлейкины, озонотерапия.

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INTRODUCTION

Mechanical trauma to the joints initiates a cascade of catabolic reactions of inflammatory origin, which are implemented mainly through the activation of lipid peroxidation processes and are accompanied by an increase in the concentration of cytokines both at the local level and in the systemic blood circulation [1, 2]. These signaling molecules form intercellular signaling pools that modify the reactivity of tissues and promote the involvement of intact tissues by primary alterations in the inflammatory process, which is achieved through the activation of lysosomal enzymes and the stimulation of the synthesis of inflammatory mediators that can increase sensitivity to oxidative stress, causing an expansion of the secondary destruction focus [3].

During the long-term pro-inflammatory potential of the joint tissues, a significant decrease in reparative processes is noted, which ultimately leads to the disorganization of the extracellular matrix and progressive disintegration of the cartilage in combination with other degenerative-dystrophic intra-articular changes [4].

As a result, the identification of the aspects of the dynamics disorders of the cytokine profile and their pathogenetic correction at the early stages enables to limit the area of secondary alteration and activate reparative mechanisms in the lesion focus, which improves the results of rehabilitation treatment, limiting the progression of post-traumatic osteoarthritis [5, 6].

AIM

The study aimed to investigate the cytokine profile in patients with post-traumatic synovitis undergoing intravenous and intra-articular ozone therapy.

MATERIAL AND METHODS

The study is based on the results of the examination and treatment of 69 patients with traumatic injuries of the knee joint, complicated by the development of post-traumatic synovitis. The patients studied were informed about the treatment and diagnostic measures and gave written consent.

Inclusion criteria were duration of trauma from one to ten days, age from 20 to 40 years, and absence of concomitant injuries and diseases.

Exclusion criteria were somatic and endocrine disorders, trauma duration > 10 days, and age < 20 and > 40 years old.

The ratio of men to women was 23:12 in group I and 22:12 in group II, respectively. Most of the patients were athletes, as well as people of working specialties. Based on the nature of the damage, patients with isolated injury of the medial meniscus, lateral meniscus, and integumentary cartilage of the articulation surface prevailed. All patients underwent medical and diagnostic arthroscopy of the injured joint. The surgical approach depended on the nature and extent of the injury.

Two study groups of patients were formed, which were similar in the volume and nature of joint injury. In the group I (35 patients), the traditional treatment was performed. A mandatory measure included the removal of the synovial-hemorrhagic punctate from the articular cavity of the damaged joint. In order to reduce inflammation and achieve an analgesic effect, non-steroidal anti-inflammatory drugs (ketorol, nimesulide, diclofenac) were used. Also, in order to increase the reparative potential of the tissues, drugs that improve peripheral blood circulation (trental, nicotinic acid, curantyl) were used, as well as physiotherapeutic treatment methods (magnetotherapy, ultrahigh frequency therapy, and laser therapy) [7].

In the group II patients (34 patients), a traditional complex therapy was supplemented with a 10-day course of intravenous ozone therapy, namely 200 ml of NaCl solution at a concentration of 2.0 mg/l once a day and intra-articular injection of 20 ml of ozone

at a concentration of 15 mg/l every other day, in the amount of five procedures. During the arthroscopy, the flow-rinsing lavage of the joint was performed with an ozonized NaCl solution at an ozone concentration of 2.0 mg/l.

An ozonator AON-01-Arz was used in the study. The study results were monitored on days 7–8 and 15–17 from the start of treatment.

The cytokine status indicators were studied, namely pro-inflammatory (TNF-α, IL-1β, IL-17, IL-6), regulatory (IL-2), anti-inflammatory (IL-4, IL-10) cytokines, as well as IL-1 receptor antagonist (IL-1RA). Blood serum was used as the test sample. The cytokines were measured by the enzyme-linked immunosorbent assay method using the Multiscan analyzer with the use of a set of reagents by Vector-Best (Novosibirsk). Peroxidase was used as an enzymatic indicator label. The mean biochemical data obtained from 20 healthy volunteers were taken as control values. The control group was comparable with the groups under study in terms of sex and age distribution. The studies were approved by the local ethical committee of the Medical Institute of the N. P. Ogarev Moscow State University (protocol No. 44 of 11/18/2012). The computational stage and statistical analysis were performed using the licensed programs SPSS-115 for Windows and Statistica 6.0.

Indicators, pg/ml	Control (n = 20)	Before treatment (n = 35)	Group I, days 7–8 (n = 35)	Group I, days 15–17 (n = 35)
TNF-α	1,20±0,02	4,51±0,19*	4,98±0,19[1]	4,39±0,31*
IL-1β	3,22±0,08	9,87±0,25*	10,24±0,31[1]	4,39±0,31*
IL-6	4,87±0,27	16,24±0,27*	17,93±0,34[1]	15,19±0,38*
IL-17	1,96±0,05	5,81±0,13*	7,03±0,34[1]	5,45±0,15*
IL-2	2,49±0,03	11,09±0,31*	12,35±0,41[1]	11,72±0,18*
IL-4	2,18±0,05	8,47±0,21*	9,97±0,33[1]	10,36±0,19*[1]
IL-1RA	468,23±18,94	1123,09±39,91*	1234,74±36,29[1]	1298,84±49,82*[1]
IL-10	7,34±0,21	23,78±0,37*	24,86±0,43	25,79±0,41*

Таблица 1. *Влияние традиционной терапии на динамику цитокинов при посттравматическом синовите*

*Примечание. * – достоверность отличия к нормальным величинам при p<0,05; [1] – достоверность отличия к данным при поступлении p_1<0,05.*

Table 1. *The effect of traditional therapy on the cytokines dynamics in post-traumatic synovitis*

*Note. * – significant difference to normal values at p<0.05; [1] – significant difference to admission data at p_1< 0.05.*

RESULTS

Analysis of the blood serum cytokine profiles upon admission of the patients showed high levels of cytokinemia. Among pro-inflammatory cytokines, TNF-α increased by 275.8% ($p < 0.001$), IL-1β increased by 206.5% ($p < 0.001$), IL-17 increased by 196.4% ($p < 0.001$); moreover, the regulatory cytokine IL-2 increased significantly by 345.4% ($p < 0.001$) (**Table 1**).

Anti-inflammatory cytokines also responded to trauma with an increase in all pools; IL-1RA increased by 139.9% ($p < 0.001$), IL-4 increased by 288.5% ($p < 0.001$), and IL-10 increased by 224.0% ($p < 0.001$) (**Table 1**). The anti-inflammatory link increased in response to an increase in the pro-inflammatory link, being a mechanism for controlling and restraining the excessive production of inflammatory mediators.

By day 7–8 of the traditional therapy, the serum cytokine content continued to increase. At the time of monitoring, when compared with the data obtained upon admission, an increase in pro-inflammatory cytokines was determined, namely TNF-α increased by 10.4% ($p_1 < 0.05$) and IL-6 increased by 10.4% ($p_1 < 0.05$), as well as the growth of IL-17 reached 21.0% ($p_1 < 0.01$) (**Table 1**). There was a further increase in anti-inflammatory and regulatory mediators, namely IL-2 increased by 11.4% ($p_1 < 0.05$), IL-1RA increased by 9.9% ($p_1 < 0.05$), and IL-4 increased by 17.7% ($p1 < 0.001$).

The continued increase in cytokines is apparently associated with the additional alteration of the joint tissues during surgery and, as a consequence, with an increase in the secretion of pro-inflammatory agents.

At the final stages of the traditional treatment, by days 15–17, the concentrations of TNF-α, IL-1β, IL-17, IL-6, and IL-2 decreased below the values recorded on admission, but did not differ significantly from them ($p_1 > 0.05$), thus signifying the emerging trend toward limiting the inflammatory process. The anti-inflammatory cytokines IL-4 and IL-1RA, in contrast to the pro-inflammatory ones, continued to increase by 22.3% ($p_1 < 0.001$) and 15.6% ($p_1 < 0.01$), respectively (**Table 1**).

The inclusion of intravenous and intra-articular ozone therapy in the treatment complex on days 7–8 did not significantly change the cytokinemia level in the blood serum relative to the results obtained with the traditional treatment ($p_2 > 0.05$), with the exception of IL-2, which decreased by 19.5% ($p_2 > 0.01$) (**Table 2**).

Indicators, pg/ml	Control (n = 20)	Before treatment (n = 34)	Group II, days 7–8 (n = 34)	Group II, days 15–17 (n = 34)
TNF–α	1,20±0,02	4,48±0,28*	5,18±0,17	**3,31±0,24***
IL-1β	3,22±0,08	9,82±0,29*	9,41±0,19	**7,65±0,16***
IL-6	4,87±0,27	16,19±0,17*	16,71±0,32	**12,14±0,26***
IL-17	1,96±0,05	5,91±0,19*	6,48±0,24	**4,51±0,23***
IL-2	2,49±0,03	11,12±0,25*	**9,94±0,34**	**8,71±0,17***
IL-4	2,18±0,05	8,54±0,13*	9,41±0,27[1]	**7,72±0,18***
IL-1RA	468,23±18,94	1118,72±36,71*	1279,63±38,59	**981,35±42,16***
IL-10	7,34±0,21	23,75±0,33*	24,92±0,43	**20,29±0,52***

Таблица 2. *Влияние комплексного применения озонотерапии на динамику цитокинов при посттравматическом синовите*

*Примечание. * – достоверность отличия к нормальным величинам при p<0,05; [1] – достоверность отличия к данным при поступлении p_1<0,05; **жирный шрифт** – достоверность по отношению к данным I группы лечения при p_2<0,05.*

Table 2. *The effect of comprehensive ozone therapy on the cytokines dynamics in post-traumatic synovitis*

*Note. * – significant difference to normal values at p<0.05; [1] – significant difference to admission data at p_1< 0.05; **in bold** – significant difference to data of Group I treatment at p_2<0.05.*

In the course of ozone application, by days 15–17, in contrast to traditional treatment, there was a synchronous decrease in all cytokine pools. The secretion of the regulatory cytokine IL-2 was inhibited to a greater extent by 25.7% ($p_2 > 0.001$). There was a decrease in the level of both pro-inflammatory TNF-α by 24.6% ($p_2 > 0.001$), IL-6 by 20.1% ($p_2 > 0.001$), IL-17 by 17.3% ($p2 > 0, 01$), IL-1β by 19.1% ($p_2 > 0.001$), and anti-inflammatory cytokines IL-4 by 25.5% (p2 > 0.001), IL-1RA by 24.4% ($p_2 > 0.001$), and IL-10 by 21.3% ($p_2 > 0.001$), relative to the values obtained with the traditional treatment (**Table 2**).

DISCUSSION

The traditional treatment course did not enable to completely arrest the systemic inflammatory process, which was confirmed by the high cytokinemia level by the end of the study; however, there was a tendency toward a decrease in the concentration of the estimated mediators, which indicates the limitation of the inflammatory activity. In addition, in the structure of the cytokine profile, a pronounced imbalance in the levels of IL-1β and the receptor antagonist IL-1RA was noted, which indicated an impairment of the regulatory mechanisms of the cytokine system and ongoing destruction of joint tissues. The active participation of the immune system under conditions of a high level of pro-inflammatory mediators and at the same time, a significant increase in IL-4 and IL-10, can contribute to the initiation of reactions underlying the development of secondary inflammation, the chronicity of the process, and the progression of destructive and degenerative changes.

Pleiotropy of the ozone therapy determines the effect on various links of pathogenesis, which determines its high efficiency in the treatment of many diseases, including those of the musculoskeletal system [8, 9]. Numerous studies describe the efficiency of medical ozone in the treatment of osteoarthritis of various origins by inhibiting matrix metalloproteases, normalization of collagen catabolism, improvement of microcirculation, and enhancing the resorption of tissue decay products, as well as correcting the antioxidant potential, resulting in a decrease in inflammatory reactions, a decrease in the damaging effect on chondrocytes and synoviocytes, and the ability to suppress the secondary alteration of the joint tissues is implemented. This is clinically confirmed by the improvement of the restorative treatment results and the reduction in the length of hospitalization [10, 11, 12].

However, when analyzing publications available for the study, there were isolated studies that assessed the cytokine profile during ozone therapy in patients with post-traumatic synovitis, and the results of its case follow-up with a combination of intravenous and intra-articular ozone administration are of significant scientific and practical value.

The results of the study showed that the use of ozone therapy in combination with the standard treatment led to a more effective correction of the cytokine profile indicators, which are markers of the pro-inflammatory potential of tissues. At the same time, a significant decrease of all cytokine pools, mainly pro-inflammatory ones, was registered, while the expression of anti-inflammatory ones was inhibited, thereby objectively confirming the suppression of the inflammatory, which contributes to the restoration of the joint function.

Ozone therapy potentiates the therapeutic effect of traditional therapy by exerting an influence on the key links of the disease pathogenesis at the systemic and regional level, which correlates with the results of studies in the world literature [6].

CONCLUSIONS

1. Knee injury initiated an imbalance of the cytokine network, thereby increasing the concentration of pro- and anti-inflammatory cytokines, which play a significant role during the post-traumatic period in the secondary alteration of the cartilage tissue and synovium, with the progression of degenerative-destructive changes.

2. Systemic intravenous and intra-articular ozone therapy effectively corrected the indicators of the cytokine profile in the post-traumatic period, thereby reducing the activity of the inflammatory reaction and limiting the formation of secondary destructive changes in the joint.

Conflict of interest. *The authors declare no conflict of interest.*

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