

Ozone improved the wound healing in type 2 diabetics via down-regulation of IL- 8, 10 and induction of FGFR expression

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Abstract. *Background and aim:* We aimed to investigate the effect of ozonated autohaemotherapy (OA) on the wound healing, serum values of interleukin (IL) - 6, 8, 10, tumor necrosis factor-alpha (TNF- α), basic fibroblast growth factor (bFGF) and local expression of fibroblast growth factor receptors (FGFR) in type 2 diabetics with the acute soft-tissue infections. *Methods:* Patients in the first cohort (n=30) received a basic comprehensive treatment (BCT-group), and the second (n=28) also received OA (OA-group). Blood samples for ELISA and tissue specimens for the immunohistochemical examinations were collected at admission (day 0) and at the 9th day of inpatient treatment. *Results:* The additional using of OA has accelerated the timing of a single and the complete wound granulation and the timing to marginal epithelization, compared with the results of the standard treatment. The use of OA has significantly reduced the production of IL-8, 10 at 9th day. OA-group patients were characterized by consistently high levels of bFGF production in contrast to the BCT-group, where the decreasing in the serum bFGF level was observed. The maximum number of bFGFR - immunopositive labels was observed in OA-group out to 9th day (319,45 (249,90-348,43) versus baseline 192,65 (171,93-207,72), versus BCT-group 123,30 (105,23- 141,10), p<0,001). *Conclusions:* Application of OA in the complex treatment of the acute soft-tissue infections in diabetics makes it possible to achieve the significant reductions in the duration of the wound inflammation and regeneration phases by eliminating of overproduction of IL- 8, 10 and induction of expression of bFGF and its receptors. (www.actabiomedica.it)

Key words: ozone, autohaemotherapy, soft-tissue infections, wound healing, diabetes mellitus type 2, basic fibroblast growth factor, fibroblast growth factor receptors

Introduction

Infections of the skin and soft tissues are a common clinical challenge with the risk of such infections post surgery high in people with diabetes mellitus due to

associated immunodeficiency, profound disturbances in the microcirculation and diabetic neuropathy (1, 2). Such localised infections create the potential for cross-infection, increase the costs of wound treatment and may support virulent strains of microorganisms within the wound (3, 4).

Normal wound healing is a well-organized process with the co-ordination between different phases under the control of growth factors and populations of platelets, fibroblasts, keratinocytes, immune surveillance, epithelial and endothelial cells (5). Of particular interest are the fibroblast growth factors (FGF) produced by keratinocytes, fibroblasts, chondrocytes, endothelial cells, smooth muscle cells, and mast cells. In the post-embryonic period FGF play the main role in the repair of skin wounds (6). Of the 23 forms of FGF fibroblast growth factor - 2 or basic fibroblast growth factor (bFGF) is among the most important, determining the formation of new blood vessels as well as influencing epidermal regeneration (7).

BFGF has several roles in cutaneous wound healing and is itself synthesised when the skin is damaged, mainly by endothelial cells and fibroblasts (8). bFGF stimulates protease secretion which helps to both destroy basal membrane vessels and to enable the migration of endothelial cells into the surrounding matrix with the subsequent formation of new blood vessels. bFGF activates the synthesis of macromolecules, in particular dermal glycosaminoglycans and hyaluronic acid, and inhibits the synthesis of collagenase-1 in melanocytes (6).

However cell responses to bFGF may be modified by the condition of its receptors (FGFR) which may explain poor healing (9, 10). The fibroblasts from chronic wounds themselves show poorer responses to growth factors than do fibroblasts from acute wounds, indicating that the fibroblasts of chronic wounds are themselves affected (11, 12). Fernández-Montequín J. I. et al. used an intralesional epidermal growth factor-based formulation in chronic diabetic foot ulcers. They showed that treatment was well tolerated and safe, but could not show improvement in healing (13). Loot M. A. and colleagues measured the response of fibroblasts from chronic ulcers to various growth factors, and compared them to fibroblasts from nonlesional controls. Fibroblasts from chronic ulcers responded poorly and needed higher doses of growth factors for the same response as healthy fibroblasts. The authors wondered if damaged receptors could be a factor in the poor response, but mentioned in their discussion that such receptor changes had been sought by others and not found (14). It is worth mentioning that epithelial

cells from chronic ulcers, like the fibroblasts, also show poor response to growth factors. However, in epithelial cells it has been possible to show a defective expression of receptors for transforming growth factor- β (15).

Systemic cytokine production is implicated in the development of insulin resistance and the disruption of glucose utilization (16) while also affecting expression of FGF and its receptors in patients with type 2 diabetes (17-19). The development of soft tissue infections may further influence the impact of cytokines on the expression of FGF and FGFR. Cytokine levels are higher in nonhealing wounds than in healing wounds, especially the levels of interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor-alpha (TNF- α) (20). The levels fell significantly when healing began to occur (21). Jiang L. et al. reported that chronic pressure ulcers displayed high levels of inflammatory cytokines, along with increased indications of apoptosis and decreased levels of growth factors (22).

The ozonated autohaemotherapy (OA) is a medical approach during which venous blood obtained from the patient is ozonated and injected back into the vein (23-26). The ozone/oxygen mixture was reported to exhibit various effects on the immune system, such as the modulation of phagocytic activity (27-29), expression of pro-inflammatory caspases (30) and cytokines in inflammation-mediated diseases (31-34). Various in vitro and in vivo studies have investigated the effect of OA on hypoxia, lipid peroxidation and antioxidant activity (35-38).

Although the ozone therapy improves the diabetic outcomes (39-42) there are few clinical studies to verify the underlying mechanisms of ozone in the wound healing in diabetic patients. Martínez-Sánchez G. et al. (2005) reported that the combined ozone treatment (local and rectal insufflation of an ozone-oxygen gas mixture) prevented oxidative stress, normalized levels of organic peroxides, activated superoxide dismutase and improved the healing of the lesions, resulting in fewer amputations (43). Topical ozone promotes the wound healing via induction of vascular endothelial growth factor, transforming growth factor- β , platelet-derived growth factor (44, 45) and by increasing the fibroblasts migration and collagen thickening (46-48). The aim of this study was to assess the effects of OA compared with standard

care in the treatment of the soft-tissue infections in patients with diabetes mellitus type 2.

Materials and Methods

Study design

This prospective non-randomized study was conducted in General Surgery Department named after Professor M. I. Gulman of Professor V. F. Voino-Yasenetsky Krasnoyarsk State Medical University (Krasnoyarsk city, Russian Federation) between December 20, 2016 and January 1, 2020. The study protocol was conformed to the ethical guidelines of the Declaration of Helsinki (2004) and approved by the Regional Ethics Committee (protocol number 42/2015). All participants provided informed consent before study enrollment.

Patients with diabetes mellitus type 2 and the acute soft-tissue infections were allocated to the first and the second cohorts. Patients in the first cohort (n=30) received a basic comprehensive treatment (BCT-group), and the second (n=28) also received OA (OA-group). Every day of inpatient treatment the clinical indicators of wound healing (the timing of disappearance of oedema and perifocal inflammation, first appearance of a separate granulation, complete filling the wounds with granulation tissue and marginal epithelization, the rate of wound healing) were assessed. Blood samples for ELISA (IL-6, 8, 10, TNF- α and bFGF) and tissue specimens for the immunohistochemical examinations (FGFR) were collected from all patients at day 0 (in surgery) and at the 9th day of inpatient treatment.

Participants

Patients with diabetes mellitus type 2 and the acute soft-tissue infections were recruited. The exclusion criteria in the study were as follows: the soft-tissue infections localized in the foot (diabetic foot); non-surgical infections, responding favorably only to conservative treatment; eczema; endotoxemia 2-3 degrees; diabetic coma; acute ischemic stroke; epilepsy; cancer; myocardial infarction; decompensated

heart failure; HIV infection; viral hepatitis; alcoholism; drug addiction; thrombocytopenia; coagulopathy; hyperthyroidism; pregnancy or nursing; patient refusal to participate in the study or involved in other studies.

Interventions

In all cases surgical intervention at admission consisted of excisional debridement, necrectomy and draining with an open wound. After the surgical debridement patients in the BCT-group received only standard treatment which included systemic antibiotic therapy, glucose management and wound sanitation by aqueous solutions of antiseptics with subsequent wound dressings appropriate for the degree of exudate and moisture maintenance.

Patients in the second cohort (OA-group) received OA in addition to standard treatment. The procedure of ozonated autohaemotherapy was carried out in line with the guidelines of the Russian Ozone Therapy Association. For OA we collected 100 ml of venous blood from a patient using a rotary peristaltic pump «Istok-2» (Istok, Russia) at a rate of 10 ml/min in a plastic container (Econika, Ukraine) containing 50 ml isotonic sodium chloride solution and 5000 ME heparin. Then 100 ml of an ozone-oxygen mixture with an ozone concentration of 10 mg/L, obtained by means of UOTA 6001 generator (Medozone, Russia), were added via an optional port in the container. During five minutes the contents of the container were carefully blended and then the blood was returned to the vein of the patient by reversing the pumping action. The dose of ozone delivered amounted to 1 mg. OA sessions were carried out from the first day of the postoperative period through days 7-9. The previous study has shown that a that using of the low doses of ozone (10 mcg/ml (total of 1 mg of ozone) is safe and have an anti-inflammatory effect in diabetics with compromised immune system (49).

Systemic antibiotic therapy in all patients with the acute soft-tissue infections on a background of type II diabetes was started by assigning third or fourth generation cephalosporins in combination with an anti-anaerobic drug (metronidazole) followed by

an antibiogram. Hyperglycaemia was corrected in all patients accordance with the recommendations of an endocrinologist.

Criteria of therapeutical effect

Every day the wound condition, presence of infection and perifocal inflammation, the need for debridement were assessed. All patients had marked the timing of disappearance of oedema and perifocal inflammation, first appearance of a separate granulation, then complete filling the wounds with granulation tissue and marginal epithelization. Wound areas were calculated daily from film transparency tracings using grid paper. The rate of wound healing was calculated by the formula: $(S - S_n) \times 100 / (S \times t)$, where S is the size of the wounds in the previous dimension, S_n —the size of the wound at the time of the study, t—number of days between the 1st and the subsequent measurement.

The serum concentrations of IL-6, 8, 10, TNF- α and bFGF were measured by using ELISA kits (Bender MedSystems, Austria) in accordance with the manufacturer's protocol. Blood samples for ELISA were collected from all patients with diabetes at day 0 and at 9th day of inpatient treatment. Each specimen was applied in duplicate.

Tissue specimens for the immunohistochemical examinations were obtained from the border area of the wound, with dimensions approximately 5 mm \times 5 mm \times 1 mm (length \times width \times depth), comprising the wound edge and surrounding skin twice: at day 0 (in surgery) and for the second time at day 9. Tissue specimens were fixed in 4% phosphate buffered formalin and paraffin embedded. Standard visualisation of FGFR involved the use of primary antibodies to FGFR-1 (the anti-FGFR-1 antibody), with dilution of 1/100 and secondary goat polyclonal anti-rabbit IgG (Cy5-labeled, Abcam, USA), with dilution of 1/200. For each slide, at least 10 fields were analyzed with high power (\times 600 magnification) microscopy by two pathologists on an Olympus CX41 CX-RFL-2 fluorescent microscopes (Tokyo, Japan). Specimens were defined as positive if there were cells distinctly stained by the antibodies. Quantitative evaluation of FGFR expression was performed using the Image J

program, Version 1.47a (National Institute of Health, USA). The number of points expressing FGFR as well as the integral intensity of the fluorescence (the product of the square of the fluorescence and the sum of the values of the pixels in relative units (r. u.) were analyzed.

Statistical analysis

Data analysis was performed with Statistical Package for Social Science software, version 22.0 (IBM, USA). Distributions of continuous and discrete numerical variables were analyzed with the Shapiro-Wilk test. The normally distributed data were presented as mean \pm standard deviation (SD), while the nonnormal distributed variables as median (Me) and 25th and 75th percentiles (LQ-UQ). The Student t-test and the Mann-Whitney U test were used for analyzing parametric and nonparametric data, respectively. Categorical variables were analyzed using the Chi-square and the Fisher's exact test. Data were evaluated at the 95% confidence interval. P-value $< 0,05$ was considered statistically significant.

Results

The cohorts of patients were comparable in terms of the nature of acute soft-tissues infections (Table. 1), the age and sex of the patients, the presence of comorbidities (prevailed arterial hypertension and obesity), and severity of diabetes (the level of glycated haemoglobin among the diabetic patients at baseline did not differ between the groups, averaging $7,93 \pm 0,53\%$, Table. 2). The median initial wound size (after the surgical debridement) in patients averaged $36,02 \pm 1,87$ cm² in BCT-group and $38,83 \pm 2,35$ cm² in OA-group ($p=0,616$).

We studied the dynamics of clinical indicators of wound healing in diabetic patients with the acute soft-tissue infections. We noted that in BCT-group, on the background of the standard treatment, long lasting signs of inflammations, time delayed granulation were presented. In OA-group the additional inclusion in complex post-operative treatment of OA allowed to achieve significant reductions in the duration

Table 1. The distribution of diabetic patients based on diagnosis

Diagnosis	The cohort of patients, abs. (%)			P
	Total	BCT-group	OA-group	
Phlegmon (upper limb, thigh, glute, inguinal, abdominal wall, thoracic wall), n (%)	18 (31,0)	7 (23,3)	11 (39,3)	0,402
Abscess (upper limb, thigh, glute), n (%)	10 (17,2)	6 (20,0)	4 (14,3)	0,732
Erysipelas, phlegmonic and necrotic forms (lower leg), n (%)	10 (17,2)	4 (13,3)	6 (21,4)	0,499
Carbuncle (back), n (%)	6 (10,3)	4 (13,3)	2 (7,1)	0,671
Traumatic wound infection (upper limb, abdominal wall, thigh), n (%)	7 (12,1)	5 (16,7)	2 (7,1)	0,425
Necrotizing fasciitis (thoracic wall, abdominal wall, inguinal), n (%)	4 (6,9)	3 (10,0)	1 (3,6)	0,612
Surgical site infection (abdominal wall), n (%)	3 (5,2)	1 (3,3)	2 (7,1)	0,605

Table 2. Demographic and clinical features of diabetic patients before treatment

Variable	The cohort of patients			P
	Total	BCT-group	OA-group	
Female, n (%)	51 (87,9)	26 (86,7)	25 (89,3)	1,000
Male, n (%)	7 (12,1)	4 (13,3)	3 (10,7)	1,000
Age in years, mean \pm SD	57,40 \pm 3,26	56,16 \pm 5,51	58,71 \pm 4,29	0,761
BMI in kg/m ² , mean \pm SD	28,16 \pm 2,13	27,52 \pm 2,91	28,83 \pm 3,11	0,705
Glycated haemoglobin in %, mean \pm SD	7,93 \pm 0,53	7,87 \pm 0,61	8,02 \pm 0,70	0,668
Comorbidity, n (%): Arterial hypertension	41 (70,7)	22 (73,3)	19 (67,9)	0,775
Over weight and obesity	40 (69,0)	20 (66,7)	20 (71,4)	0,780
Chronic obstructive pulmonary disease	10 (17,2)	6 (20,0)	4 (14,3)	0,732
Cerebrovascular disease	7 (12,1)	3 (10,0)	4 (14,3)	0,701

Table 3. Comparison of clinical indicators of wound healing

Indicators	BCT-group	OA-group	P
The timing of disappearance of oedema and perifocal inflammation in days, mean \pm SD	9,17 \pm 1,22	5,81 \pm 1,33	0,001
The timing of appearance of a single wound granulations in days, mean \pm SD	11,23 \pm 0,90	7,02 \pm 0,94	<0,001
The timing of emergence of marginal wound epithelisation in days, mean \pm SD	14,94 \pm 1,81	10,62 \pm 1,15	0,001
The timing of the complete wound granulation in days, mean \pm SD	18,61 \pm 2,07	12,49 \pm 1,90	0,001

of the inflammation (till 7,02 \pm 0,94 days) and regeneration phases (till 12,49 \pm 1,90 days) compared with the same indicators in BCT-group (11,23 \pm 0,90 and 18,61 \pm 2,07 days, respectively, $p \leq 0,001$, Table. 3, Fig. 1). The rate of wound healing in inflammation phase averaged in BCT-group and OA-group 1,51 \pm 0,11%, and 1,67 \pm 0,15% respectively ($p=0,118$); in regeneration phase – 2,84 \pm 0,18% and 5,81 \pm 0,22% respectively ($p < 0,001$).

Laboratory findings indicated all diabetic patients experienced moderate leukocytosis (within 12,75 \pm 1,51 x 10⁹/l). The cytokines level in the blood of patients with diabetes mellitus in the different cohorts did not differ significantly at baseline (Table. 4). On the 9th day of inpatient treatment, average levels of IL-6 in BCT and OA-groups had decreased by 1,69 and 3,04-fold, respectively, reaching their minimum in OA-group (0,53 (0,19-0,72) ng/ml, $p=0,194$).



Figure 1. Necrotizing fasciitis of the abdominal wall in OA group female: 1 - status at baseline (extensive soft tissue necrosis involving the skin of the abdominal wall with purulent discharge), 2 - at surgery (excisional debriding and necrectomy with an open wound), 3 - at day 6 (disappearance of purulent discharge and perifocal inflammation), 4 - at day 13 (the complete wound granulation and marginal epithelisation), 5 - at day 14 (after covering the remaining soft-tissues defect by the secondary sutures), 6 - at day 96 (after discharge).

There were no significant changes in the level of IL-8 out to 9th days in BCT-group (24,96 (12,36-30,67) pg/ml versus 25,30 (12,47-26,03) pg/ml at baseline, $p=0,792$). The additional use of the systemic ozone therapy reduced the concentration of IL-8 by 3,84-fold in comparison with the initial value (6,82 (3,42-13,90) pg/ml versus 26,17 (12,58-28,94) pg/ml, respectively, $p<0,001$). The achieved rate differed significantly from those in BCT-group (24,96 (12,36-30,67) pg/ml, $p<0,001$).

Against the background of the complex treatment the level of anti-inflammatory IL-10 decreased in all diabetics but most significantly in OA-group (0,35 (0,17-0,88) pg/ml versus baseline (2,99 (1,42-4,52) pg/ml), $p<0,001$), versus BCT-group (0,91 (0,41-1,32) pg/ml), $p=0,028$, (Table. 4).

All patients with diabetes mellitus type 2 and the acute soft-tissue infections were marked by similar systemic levels of TNF- α at admission, which then tended to decline. The differences, achieved in the different cohorts out to day 9 of inpatient treatment had no statistical significance (Table. 4).

The serum level of bFGF in diabetics with the acute soft-tissue infections did not differ significantly

in the different cohorts at baseline (Table. 4). In the postoperative period in BCT-group the decreasing in the serum bFGF level was observed (17,51 (14,93-19,83) pg/ml versus 24,11 (18,65-33,16) pg/ml, $p=0,022$), in contrast to the OA-group of patients, who were characterized by consistently high levels of bFGF production (25,53 (20,35-27,44) pg/ml versus 23,84 (17,30-28,33) pg/ml, $p=0,334$).

In evaluating the results of immunohistochemical analysis of all patients with diabetes mellitus type 2 and the acute soft-tissue infections were observed a similar value of the absolute number of FGFR- immunopositive labels at baseline (Table. 5). Against the background of the standard therapy patients in BCT-group continued to show suppression of FGFR expression (123,30 (105,23-141,10) – 1,6 fold in comparison with the initial number (195,14 (149,81-237,86), $p=0,001$), with a simultaneous increase in the intensity of the fluorescence (0,66 (0,55-0,72) r. u. versus 0,45 (0,39-0,52) r. u. at baseline, $p<0,001$).

In OA-group there were increases in the number of FGFR-immunopositive labels out to 9th day by 1,7 fold (319,45 (249,90-348,43) versus 192,65 (171,93-207,72) at baseline, $p<0,001$). The achieved rate

Table 4. Cytokines levels at different time points

IL	Day 0			Day 9		
	BCT-group	OA-group	P	BCT-group	OA-group	P
IL-6 in ng/ml, Me (LQ-UQ)	1,32 (0,81-2,49)	1,61 (1,14-3,89)	0,540	0,78 (0,22- 1,32)	0,53 (0,19- 0,72)	0,294
IL-8 in pg/ml, Me (LQ-UQ)	25,30 (12,47-26,03)	26,17 (12,58- 28,94)	0,816	24,96 (12,36-30,67)	6,82(3,42- 13,90)	<0,001
IL-10 in pg/ml, Me (LQ-UQ)	2,81 (1,96-3,89)	2,99 (1,42- 4,52)	0,738	0,91 (0,41-1,32)	0,35 (0,17-0,88)	0,028
TNF- α in ng/ml, Me (LQ-UQ)	2,06 (1,32- 2,49)	1,93 (1,08-3,63)	0,839	2,02 (1,05-2,27)	1,51 (0,85-2,53)	0,479
bFGF in pg/ml, Me (LQ-UQ)	24,11 (18,65-33,16)	23,84 (17,30- 28,33)	0,920	17,51 (14,93- 19,83)	25,53 (20,35-27,44)	0,001

Note: IL – interleukin, TNF- α - tumor necrosis factor-alpha, bFGF - basic fibroblast growth factor

Table 5. Fibroblast growth factor receptors expression in tissues

Indicator	Day 0			Day 9		
	BCT-group	OA-group	P	BCT-group	OA-group	P
number of FGFR positive labels per 0,332 mm ² , Me (LQ-UQ)	195,14 (149,81-237,86)	192,65 (171,93-207,72)	0,810	123,30 (105,23- 141,10)	319,45 (249,90- 348,43)	<0,001
fluorescence intensity of FGFR positive material in r. u, Me (LQ-UQ)	0,45 (0,39-0,52)	0,40 (0,34-0,57)	0,102	0,66 (0,55-0,72)	0,62 (0,49-0,69)	0,688

Note: FGFR - fibroblast growth factor receptors, r. u - relative units

differed significantly from that of BCT-group ($p < 0,001$, Table. 5). The fluorescence intensity of FGFR-positive material out to 9th day of inpatient treatment in OA-group was increased, but did not differ significantly from that of BCT-group ($p = 0,688$, Table. 5).

Discussion

These results confirm the literature data on cytokine imbalance as one of the key factors that inhibit repair processes in diabetes mellitus. It's known, that diabetes associate with absence of an acute inflammatory response important for wound healing progression and instead reveal a persistent inflammation throughout the healing process (50). Dasu MR, Martin SJ. showed that increased toll-like receptors expression, signaling, and activation may contribute to the hyperinflammation in the human diabetic wounds (51). Dinh T. et al. reported that increased levels of inflammatory cytokines were observed in serum specimens in diabetic patients taken at the baseline visit, which occurred on average 8 months before the development of foot ulceration. Therefore, they emphasized, that raised levels cannot be attributed to mechanisms that are related with the healing process of an existing ulcer and clearly indicate that a pre-existing proinflammatory state has a negative impact for the wound healing and can lead to resistance of the growth factor action (52).

We noted that in BCT-group, on the background of the standard treatment, compared with additional using of OA, long lasting IL-8 overexpression was presented. Hyperinflammation favours wound matrix degradation, thus, amplifying a pre-existing granulation tissue productive cells' invasiveness and recruitment deficit (53). Proinflammatory cytokine hyperexpression perpetuates homing of inflammatory cells, triggers proapoptotic genes and impairs reepithelialisation (54). The continued presence of the wound as a result of cytokine-mediated inhibition of repair processes is complementary, along with impaired microcirculation and risk of local hypoxia for microbial persistence and superinfection, which in turn is an incentive to the production of inflammatory mediators and closes the "vicious circle" of the pathogenesis of non-healing wounds (55).

Various therapeutic approaches successfully applied to improve the processes of wound healing on

the background of diabetes, aimed to downregulate proinflammatory cytokine hyperproduction (56, 57). The ozone/oxygen mixture was reported to normalize the expression of pro-inflammatory caspases (30) and cytokines in inflammation-mediated diseases (31-34). The treatment with increasing doses of ozonated serum was found to activate the nuclear factor-erythroid-2-related factor 2 in a dose dependent manner and to induce the expression of heme oxygenase-1 and nicotinamide adenine dinucleotide phosphate quinone oxidoreductase-1 in endothelial cells (58). The major auto-hemotherapy by low ozone doses in healthy volunteers increased the levels of Nrf2 in peripheral blood mononuclear cells with consequent enhanced activity of superoxide dismutase and catalase (59). Treatment with the OA in diabetic patients with the soft tissues infections has been shown to eliminate the overexpression of IL-6, 8 and 10 (49). In present study the use of OA has significantly reduced the production of IL-8, 10 and increased the bFGF expression at 9th day compared with the same indicators of BCT-group.

One of the most resistant mechanisms regulating the wound healing in patients with type 2 diabetes is FGFR expression which was marked by initially low values in patients in BCT-group and continued to progressively decrease out to 9th postoperative day. It's known, that fibroblast growth factors exert their effects through the transmembrane high-affinity fibroblast growth factor receptors and in this way, regulate cell proliferation, differentiation, and function in a number of tissue processes, including normal development, carcinogenesis and metastasis (60). The expression of FGFs and its receptors was found altered in diabetic tissues compared to normal ones in various tissues including skin, human placenta and adipose tissue (61-63). Kaftan H. et al. used a specific inhibitor of the FGFR tyrosine kinase for creation an animal model of chronic tympanic membrane perforation and reported that tympanic membrane healing was delayed in a dose-dependent manner (64). Galkowska H. et al. analyzed the expression of various chemotactic and growth factors and their receptors in the margin of diabetic foot ulcers and in normal non-diabetic skin. They found lack of up-regulation of bFGF and its receptors in the ulcer margin and suggested that it associated with the reduced influx of immune cells, may account for a poor formation of granulation tissue and chronicity of ulcer epithelialization (61).

It's known, that noninvasive oxygen-ozone therapy promotes the wound healing in diabetic patients with foot ulcers via potential induction of vascular endothelial growth factor, transforming growth factor- β and platelet-derived growth factor (65). Soares C. D. et al. showed that bFGF immunoreactivity was significantly higher in wounds treated with subcutaneous ozone injection compared to controls (66). The OA combined with noninvasive oxygen-ozone therapy was reported to increase the FGFR expression in diabetic patients with the soft-tissue infection (67). In our study, the additional use only of OA in the treatment of the acute soft-tissues infections in patients with diabetes mellitus has increased the absolute number of FGFR- immunopositive labels in the wounds at the 9th day of the postoperative period compared to the standard treatment. The results show that the efficacy of the OA treatment for the wound healing in diabetics may be due to the eliminating overproduction of IL- 8, 10 and induction of expression of bFGF and its receptors, which has not been reported before.

We have not observed any negative effects or complications from conducting OA, including those described in the literature cases of hemorrhage or venous thrombosis and embolism (68), possibly in connection with the exclusion from the study patients with contraindications to ozone therapy.

Our study has some limitations. First, our study is a non-randomized single-center study, the second limitation is the small number of cases, and the last limitation is the short follow-up period limited by the times of inpatient treatment.

Conclusion

Application of OA in the complex treatment of the acute soft-tissue infections in patients with type 2 diabetes makes it possible to achieve the significant reductions in the duration of the wound inflammation and regeneration phases by eliminating of overproduction of IL- 8, 10 and induction of expression of bFGF and its receptors.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Informed consent: Written informed consent was obtained from patient, and the study was approved by the ethics committee of the institution.

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