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EFFECT OF A COMPLEX ACTION OF OZONETHERAPY COMBINED WITH AUTOLOGOUS PLATELET-RICH PLASMA APPLICATION ON THE CHARACTER OF MORPHOFUNCTIONAL CHANGES OF THE TISSUE MACROPHAGES IN PATIENTS WITH DIABETIC FOOT SYNDROME

Introduction

Macrophages play a considerable role in the process of activation of inflammatory process mechanisms in the wound [1-3]. At the expense of exocytosis by phagocytes (neutrophils and macrophages) in the wound toxic products of tissue decay are accumulated, the level of various enzymes and other biologically active substances increases, promoting death not only of microorganisms but healthy cells as well [4, 5]. This especially applies to patients suffering from type 2 diabetes mellitus (DM) with the signs of macro- and microangiopathy of vessels of the lower limbs. At the final stage of the course this process is manifested by critical ischemia, slow wound healing and necrosis of the soft tissues [6, 7].

Objective

To study the character of ultrastructural changes in the tissue macrophages under the effect of a complex of auxiliary factors promoting reparative process activation in the wound including ozonotherapy, local application of autologous platelet-rich plasma (PRP) and vacuum therapy.

Materials and Methods

For the period from 2018 to 2021, 70 patients with purulent-necrotic process and ischemic-gangrenous form of diabetic foot syndrome were examined at the Surgical Department №1, Regional Municipal Non-Profit Institution “Chernivtsi Emergency Hospital”, the Department of Surgery-2, Bukovinian State Medical University. Surgery on all the patients was performed with maximum possible saving of the vessels in the plantar region. The research involved 41 (58,57%) men and 29 (41,42%) women. The majority of patients (56 individuals) were over the age of 65-67 years (80%). All the patients suffered from type 2 DM. The distribution of patients according to their number and age was identical and did not differ reliably (p > 0.05) (Table 1).
The patients examined were divided into three groups. The first group included 23 (32,85%) patients who were treated by means of regional ozone therapy. The second group included 23 (32,85%) patients treated by means of local PRP therapy. The control group included 24 (34,28%) patients who during the postoperative period received a complex of therapeutic measures according to the generally accepted method [8].

The first group examined was given intravenous infusions with ozonized physiological solution (OPS). Ozone concentration in the intravenous infusion solution was 2,0–2,5 mg/L, and it was administered at a rate of 40–60 drops per minute. At a nearly postoperative period the number of procedures performed was six, and it depended on the dynamics of the disease and local changes in the place of foot affliction [9, 10].

The second group examined, 24 hours later after surgery performed, received a complex of therapeutic measures complemented by the method of local intra-tissue PRP administration in the total volume under 5,0 ml. The amount of 0,5 ml was injected subcutaneously at a distance of 1,5 cm from the wound edges, and with open administration 2,5 ml of the amount—in the areas of predicted formation of the granulation tissue [11, 12].

The control group received a complex of therapeutic measures according to the standard requirements [13-15].

Electronic-microscopic examination used the fragments of the wound surface tissue preserved in 2,5% glutaraldehyde solution on phosphate buffer (pH − 7, 2-7,4), followed by further processing in 1% anhydride solution. The fragments were dehydrated in increasing concentration alcohols, and then placed into araldite. The morphological structures were contrasted with a concentrated solution of uranyl acetate in the process of dehydration, and on the sections — with lead acetate. The sections 40-60 nm thick obtained by means of ultratome УМТП-3 were examined under the electron microscope TESLA БС-500. The samples of biological material were taken from patients when dressing on the 7th, 14th and 2–23rd days of treatment. A generalized percentage assessment of the dynamics of changes in the tissue macrophages was carried out according to the Astaldi principle, a semi-quantitative method considering distinctive cytological changes determined in the similar amount of the cells examined (tissue macrophages).

### Results and discussion

Stimulation of the macrophage functional activity under ozone effect with destructive changes found in the cells without necrotizing damage was found to be explained by apoptosis mechanism involvement as a positive factor in the regulation of local homeostasis on completion of inflammatory (exudative) phase of the wound process. Such type of dynamics of changes in the tissue macrophages functional activity was determined on the 14-18th day of the postoperative period (Fig. 1).

![Fig. 1. 21-23 day of the postoperative period— the first group examined.](image1)

Activation of phagocytic possibilities of the tissue macrophages with local application of autologous platelet-rich plasma occurs without any signs of apoptosis prevalence. It is indicative of stimulation of their functional activity by antigenic or enzymatic mechanisms stimulating their functional activity (Fig. 2, 3).

![Fig. 2. 21-23 day of examination (in the second group examined). Complete phagocytosis. Residual bodies of microorganisms and tissue structures present.](image2)
Fig. 3. 21-23 day of examination (in the second group examined). Transverse section of the external cellular membrane in vagination with present intracellular structures and signs of active macrophage phagocytic activity. Electronogram, ×15000

Fig. 4. 21-23 day of examination (control group). Incomplete phagocytosis. Certain microorganisms at the stage of division. Electronogram, ×45000

Fig. 5. 21-23 day of examination (control group). Formation of vacuoles at the sites of destructively changed mitochondria. Electronogram, ×20000

Fig. 6. 21-23 day of examination (control group). Macrophage fragment with signs of destruction of the cellular membrane structures. Electronogram, ×25000

Table 2

Dynamics of microstructural and functional changes in the tissue macrophages in patients with IV degree of ischemic process and diabetic foot syndrome according to Meggitt-Wagner classification. (Astaldi principle, a semi-quantitative method)

<table>
<thead>
<tr>
<th>Distribution of the groups of patients</th>
<th>Microstructural changes in the tissue macrophages (main group)</th>
<th>7 day</th>
<th>14 day</th>
<th>21-23 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>The 1st group examined. (ozone therapy) 23 (26.66%)</td>
<td>Absence of phagocytosis, Extracellular lysis of the tissue structures, vacuolization of the cytoplasmic macrophage complex</td>
<td>82 ± 8 % (-)</td>
<td>44 ± 9 % (+)</td>
<td>74 ± 12 % (++)</td>
</tr>
<tr>
<td>Phagocytic activity</td>
<td>Incomplete phagocytosis, vacuolization of the cytoplasmic macrophage complex</td>
<td>Partial restoration level of the macrophage phagocytic activity</td>
<td></td>
<td></td>
</tr>
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<td>Distribution of the groups of patients</td>
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<td>7 day</td>
<td>14 day</td>
<td>21-23 day</td>
</tr>
<tr>
<td>The 2nd group examined. (local PRP application) 23 (26.66%)</td>
<td>Absence of phagocytosis, Extracellular lysis of the tissue structures, vacuolization of the cytoplasmic macrophage complex</td>
<td>84 ± 14 % (-)</td>
<td>38 ± 11 % (+)</td>
<td>62 ± 9% (++)</td>
</tr>
<tr>
<td>Phagocytic activity</td>
<td>Incomplete phagocytosis, vacuolization of the cytoplasmic macrophage complex</td>
<td>Partial restoration level of the macrophage phagocytic activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 24 (24.76%). (a complex of therapeutic measure carried out according to the generally accepted method)</td>
<td>Microstructural changes in the tissue macrophages (control group)</td>
<td>87 ± 11 % (-)</td>
<td>34 ± 12 % (+)</td>
<td>54 ± 14 % (+)</td>
</tr>
<tr>
<td>Phagocytic activity</td>
<td>Absence of phagocytosis, Extracellular lysis of the tissue structures, vacuolization of the cytoplasmic macrophage complex</td>
<td>In complete phagocytosis, vacuolization of the cytoplasmic macrophage complex</td>
<td>Partial restoration level of the macrophage phagocytic activity In complete phagocytosis, vacuolization of the cytoplasmic macrophage complex</td>
<td></td>
</tr>
</tbody>
</table>
Degenerative-swelling changes in these cells prevailed in patients from the control group on the 21-23rd day (Fig. 4-6).

A great part of mitochondria had marked signs of destruction and formation of multiple vacuoles (Fig. 5).

The process of swelling of the macrophage microstructures with illumination of the homogenized matrix and determined vacuolization of mitochondria dominated in the samples of the material examined. The cytoplasmic matrix and were practically devoid of cristae. Examination of tissue macrophages in the control group on the 21st day found the signs of incomplete phagocytosis (Fig. 6, table 2).

Based on the data in the table, it can be seen that the patients of the studied groups have a higher partial level of restoration of the phagocytic activity of the macrophage, both on the 14th and on the 23rd day of treatment. Whereas in patients of the control group, incomplete phagocytosis and vacuolization of the cytoplasmic complex of macrophages remained until the 23rd day.

What this shows is that the use of various factors to stimulate the phagocytic activity of macrophages improves reparative possibilities in the treatment of the purulent-necrotic process in diabetic foot syndrome.

These results indicate differences in microstructural changes and restoration of macrophage phagocytic activity in the ozone therapy group, the topical PRP group, and the control group over the observed time periods.

**Conclusions**

1. Stimulation of the macrophage functional activity under ozone effect and destructive changes available in the cells without necrotizing damage can be explained by involvement of apoptosis mechanism as a positive factor in the regulation of local homeostasis on completion of inflammatory (exudative) phase of the wound process.

2. Activation of phagocytic possibilities of the tissue macrophages with local application of autologous platelet-rich plasma occurs without any signs of apoptosis prevalence. It is indicative of stimulation of their functional activity by antigenic or enzymatic mechanisms stimulating their functional activity.

3. A complex of such auxiliary therapeutic measures as regional ozone therapy, vacuum sanitation and local application of autologous platelet-rich plasma (PRP) confirm more determined level of the reparative process activation on the level of the tissue macrophages in both groups examined (№ 1 and № 2) in comparison with the control one.

**Prospects of further research.** The results obtained confirm and determine further necessity to carry out search in novations in the treatment to diabetic foot syndrome using the factors of physical and biological effect directed to the activation of the wound reparative process.

**ПЕРЕЛІК ПОСИЛАНЬ**


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