

DETERMINATION OF OPTIMAL OZONE DOSES ON THE BASIS OF THE ASSESSMENT OF HEMOSTASIS SYSTEM PARAMETERS IN BURNED PATIENTS

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The aim of the investigation was to assess the effect of clinical ozone doses on blood coagulation in the treatment of burned patients.

Materials and Methods. The control group had 34 of apparently healthy people. The comparison group consisted of patients receiving standard infusion-transfusion therapy used in clinic unit of thermal injuries (n=58). The study groups included the patients with burn disease, who underwent an ozone therapy course of five ozone procedures against the background of conventional treatment methods. They were injected a single dose of ozone intravenously in the form of ozonized saline solution: 40–80 µg (n=10), 120–160 µg (n=10), 200–250 µg (n=13), and 500 µg (n=16). The hemostatic system condition was studied by the following parameters: thrombin time, prothrombin time, activated partial thromboplastin time, fibrinogen concentration, antithrombin III activity.

Conclusion. The burned patients under the influence of ozone therapy and depending on ozone dose showed varying degrees of marked changes in the hemostatic system. The most optimal dosage affecting the blood coagulation system appeared to be the dose of 120–160 µg promoting the reduction of fibrinogen concentration and increased antithrombin III activity.

Key words: burn disease; hemostatic system; ozone therapy.

Modern analysis of the development of alteration shifts in burn injury includes the consideration of hemostatic system abnormality as one of primary factors of burn disease pathogenesis precipitating the course of the disease [1, 2]. The breakdown of blood clotting system mechanisms in burn injury is a patent example of the so-called vicious circle in burned patients, when the resultant complication sharply exacerbates the course of the underlying disease due to microcirculation abnormality, hypoxia development, and depression of functions of the most important internal organs. So, in the liver there is the abnormality of biosynthesis of not only bioactive compounds participating in various metabolic process of the body, but also those, which are directly related to the realization of mechanisms of clotting, fibrinolysis, and endogenous anticoagulants.

Literature data analysis showed there is strong interrelation between free radical oxidation and hemostatic system [3, 4]. There are known dose-dependent ozone effects relating to hemostatic system manifesting both in hypocoagulation and hypercoagulation [5–8]. These facts are of special interest in ozone application in the management of burn disease, which is accompanied by hemostatic system changes.

Moreover, literature data analysis showed the lack of the information on the effect of various concentrations of

ozonized normal saline solution injected parenterally on hemostatic system of burned patients.

The aim of the investigation was to assess the effect of clinical ozone doses on blood coagulation in the treatment of burned patients.

Materials and Methods. The research subject of hemostatic system was citrated blood of patients with burn area of 15% and more. The control group consisted of 34 of virtually healthy people. The experimental group included patients receiving standard infusion-transfusion therapy used in a clinic unit of thermal injuries (n=58). The study groups consisted of patients with burn disease, who underwent an ozone therapy course of five ozone procedures against the background of conventional treatment methods. They were injected ozone intravenously in the form of ozonized saline solution, a single dose being: 40–80 µg (n=10), 120–160 µg (n=10), 200–250 µg (n=13), and 500 µg (n=16). The blood was withdrawn on day 1–4 (after 1–2 ozone therapy procedures) and on day 10–15 after injury (at the end of ozone therapy treatment). The hemostatic system condition in the patients was studied by the following parameters: prothrombin time, thrombin time, activated partial thromboplastin time (APTT), fibrinogen concentration. Endogenous anticoagulants were estimated by antithrombin III (AT III) activity.

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Results and Discussion. The sufferers with burn injuries in the experimental group had 14% increase of fibrinogen concentration compared to the norm, starting from as early as the first days after injury (Table 1). By day 10–15 fibrinogen level increased by 71% in comparison with the same parameter in healthy people. Enhanced high-molecular fibrinogen concentration increases thrombosis risk, blood viscosity. Fibrinogen is known to be extremely sensitive to reactive oxygen species (ROS) [9]. Oxidized fibrinogen concentration grows after burn injury, against the background of increased intensity of free radical processes [10]. There is evidence that oxidatively modified fibrinogen depending on its concentration and oxidation level can have an effect on blood coagulation factors, platelet and red blood cell aggregation [10–12].

We have found that after 1–2 ozone therapy procedures there are no statistically significant changes in fibrinogen level compared to the similar index in patients without ozone therapy. However, there is the tendency for the increase of this protein concentration due to the administered ozone dose (See Table 1).

The study of fibrinogen content in the groups after ozone therapy showed ozone dose of 120–160 µg to have an optimizing effect on fibrinogen level, and the index was found to decrease by 31% by the end of the treatment course compared to similar parameter in the experimental group. Fibrinogen concentration reduction under ozone can be related to the stimulation of tissue type plasminogen activator oxidated by fibrinogen [13]. Plasmin substrates are not only fibrins forming a clot, but also some blood clotting factors including fibrinogen. Thus, reducing fibrinogen concentration, including fibrinogen oxidated initially and that resulted from prooxidant therapy, ozone dose of 120–160 µg promoted blood coagulability reduction, and contributing

by that the improvement of its rheological properties, and therefore, improving blood supply and trophism of organs and tissues.

40–80 µg dose of ozone had no marked effect on fibrinogen content. As well as in a group of patients without ozone therapy, in the course of burn disease there was further fibrinogen content growth (by 52%). Similar changes of hyperfibrinogenemia were revealed when other doses of ozone were administered: 200–250 µg of ozone rose fibrinogen content by 34%, dose of 500 µg — by 16% in comparison with fibrinogen level on day 1–4 after injury (See Table 1).

To assess external mechanisms of plasma hemostasis, we determined prothrombin time characterizing prothrombin complex during thrombin formation. Its mean value in all groups of patients at the beginning and at the end of the observation period was found to be within the range of reference values (12.9–18.5 s).

In all examined patients thrombin time characterizing the rate of fibrinogen transformation into fibrin slightly differed from arithmetic mean value in healthy people (15.2–0.15 s) within the range of normal limits throughout the observation period regardless of ozone dose. An exception was the tendency for hypocoagulation revealed using saline solution with ozone dose of 120–160 µg by the end of ozone therapy course: averaged thrombin time increased by 14%. The mechanism of such ozone effect can be associated with ROS modified effect on proteins of blood coagulation system. ROS, as well as oxidized fibrinogen can act not only as hemostasis activating factors, but also have an inhibiting effect on clotting cascade, as they have phospholipid structure, due to which they are oxidated easily.

Activated partial thromboplastin time is a test responsive to coagulation defects, especially to deficiency of XII, XI, IX and VIII factors providing internal clotting mechanisms. We have found APTT in burned patients of the control group and all the patients receiving ozone therapy to have no significant differences from arithmetic mean of the index (39.90–0.61 s) and be in the limits close to normal during all observation periods.

The condition of blood protective system against hypercoagulation progression was estimated by antithrombin III activity accounting for 75–80% of all anticoagulant blood potential. AT III is an inhibitor of thrombin, Xa, IXa, XIa, XIIa factors. The findings indicate the decreased AT III activity resulted from burn injury as early as on day 1–4 after injury compared to the level of this index in virtually healthy people (Table 2) that is in compliance with the information recorded in literature [14]. In these periods intravenous ozone administration had no effect on the activity of this anticoagulant.

After ozone therapy we revealed no significant alterations in AT III activity using a single ozone dose of 40–80 µg. The patients who had received ozone at a dose of 120–160 µg were found to have significant AT III activity increase — by 32% in comparison with that at the beginning of the course, and by 21% compared to the value of the parameters in the experimental group on day 10–15 after burn injury. The more evident and statistically significant increase of AT III

Table 1
The changes of fibrinogen content under ozone in burned patients, g/L (M±m)

	Groups of patients	
	Day 1–4 after burn (after 1–2 ozone therapy procedures)	Day 10–15 after burn (after ozone therapy course)
Healthy people (control group)	2.80±0.11	
Experimental group	3.20±0.11	4.800±0.151*
40–80 µg of ozone	2.90±0.17	4.40±0.43**+
120–160 µg of ozone	3.30±0.33	3.30±0.34#v°
200–250 µg of ozone	3.50±0.39	4.70±0.22^
500 µg of ozone	3.70±0.33	4.30±0.28

Note: * — p=0 compared to the experimental group on day 1–4; ** — p<0.05 compared to fibrinogen concentration early in the ozone (dose of 40–80 µg) therapy; + — p<0.05 compared to fibrinogen level on day 10–15 using ozone at a dose of 120 µg; # — p<0.01 compared to the experimental group on day 10–15; v — p<0.001 compared to fibrinogen level on day 10–15 using ozone dose of 200 µg; ° — p<0.05 compared to fibrinogen level on day 10–15 using ozone dose of 500 µg; ^ — p<0.01 compared to fibrinogen content at the beginning of ozone therapy using ozone dose of 500 µg.

Table 2

The effect of different ozone doses on AT III activity in early burn disease, % (M±m)

Groups of patients	Day 1-4 after burn (after 1-2 ozone therapy procedures)	Day 10-15 after burn (after ozone therapy course)
Healthy people	97.10±2.02	
Control group	68.90±1.69	74.60±2.05
40–80 µg of ozone	71.70±4.13	77.70±8.23
120–160 µg of ozone	71.50±8.11	94.50±2.63 ^{*+v}
200–250 µg of ozone	70.50±1.67	109.70±10.31 ^{#^}
500 µg of ozone	71.70±4.63	82.10±3.18 [°]

Note: * — p<0.05 compared to AT III activity at the beginning of ozone therapy; + — p<0.05 compared to the experimental group on day 10–15; v — p<0.05 compared to AT III activity on day 10–15 using ozone dose of 500 µg; # — p<0.01 compared to AT III activity at the beginning of ozone therapy course; ^ — p<0.005 compared to the control on day 10–15; ° — p<0.01 compared to AT III activity at the beginning of ozone therapy.

activity was recorded when using ozone dose of 200–250 µg at the end of the observation period (by 55% — compared to the level of the studied parameter after 1–2 ozone therapy procedures, and by 44% — in comparison with the parameter value in patients without ozone therapy). Such AT III activity changes can be associated with a corrective ozone effect on a protein synthesizing function of the liver [15], where this anticoagulant is synthesized. Since AT III is the most powerful thrombin inhibitor, which transforms fibrinogen into fibrin microthrombi, the increase of AT III activity resulting in the reduction of microthrombus formation is to be estimated as an essential therapeutic result of ozone therapy.

Less expressed AT III activity using ozone dose of 500 µg can be due to the fact that the increased amount of ROS and lipid hydroperoxides in ozone administration is accompanied by an increased AT III consumption that in its turn can promote thrombus threatening condition.

Based on the survey, it may be concluded that the application of parenteral method of ozone therapy in the management of burned patients is accompanied by alterations in hemostatic system depending on ozone concentrations in saline solution. An oxidative effect of reactive oxygen species results in the shifts in functional condition of this system, and plays a key role in adaptation of the organism, particularly in the system of endogenous blood anticoagulants. When administering systemic ozone therapy in burn disease one should take into account the intensity of disorders in hemostatic system.

Conclusion. The burned patients under ozone therapy and depending on ozone dose showed varying degrees of marked changes in the hemostatic system. The most optimal dosage affecting the blood coagulation system appeared to be the dose of 120–160 µg.

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