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review

Ozone therapy in traumatology and burns treatment

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Abstract

This review summarizes the bases of the use of ozone in wound healing in patients suffering from trauma or burns. Different clinical assays had shown that local or systemic application of oxygen-ozone therapy in wounds and superficial scalds results in accelerated cleansing of a wound from necrotic tissues without generate necrosis. In addition to the well know germicide effect of ozone, its application can stimulate the local tissue regeneration, the local immune response and can control the inflammatory reaction. The quick management of pain and edema, the reduction of epithelialization time and the better graft retention are part of the benefit of the integrative use of ozone in traumatology and burns treatment.

Bases on the Russian clinical experience the indication, contraindication, protocols and specific care during the application of this therapy was described. The systemic ozone therapy conducted after an analysis of the oxidative stress status in those patients was referred. The concept of integration of ozone therapy as part of the routing care of the wounds is expressed. In this context the application of ozone offer many advantages, for instance: ozone reactivating the oxygen homeostasis, can increase the antioxidant activity of blood plasma, improve peripheral circulation and microcirculation, induce homeostasis compensation, and modulate the anti-inflammatory and immune response in addition to their detoxification effects

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Introduction

The application of oxygen-ozone mixture in traumatology and combustiology was approved by the Russian Ministry of Public Health (№ ФС- 2007/029-У) in 2007. The approved protocols include the methods of the local and systemic use of ozone and ozonized solutions for patients with injured, burnt associated with wound dystrophy, burn disease, septic heard-to-heal wounds or osteomyelitis. The recommended methods (the irrigation with a gas mixture in plastic bag under normal pressure or in the hypo and hyperbaric mode, the use of antiseptic ozonized solutions - distilled water, normal saline solution - the subcutaneous, intramuscular, intra-articular ozone administration, vacuum-vibration massages, ozonized baths, rectal oxygen-ozone mixture insufflations, the local combined use of ozone with absorbents, silver-containing preparations and the intravenous administration of ozonized saline solution) may possible to handle this pathology more effectively, extend the range of aids for the conservative regular therapy, give chance to obtain positive clinical results and multiply chances for the recovery of patients.

The urgency of the local and systemic application of ozone in case of burn disease and wound dystrophy is caused by antibacterial-resistant microbial flora, by endotoxicosis, by concurrent local and generalized infection or by progressive multisystem organ failure. The scientific bases of the use of ozone therapy in burns are associated with the high oxidation capacity of ozone accompanying the antibacterial, virucidal, fungicidal action and furthering the oxidative detoxication of the organism of the injured patient.

In the complex treatment of this pathology over 1200 patients were exposed to oxygen-ozone therapy. Among them there were injured persons with the II-III or IV degree burns of affected area from 10 % to 85%, with posttraumatic, postsurgical and gunshot osteomyelitis, with heard-to-heal infected wounds.

At the level of the whole organism of burnt persons, the physiologic and curative effects of ozone are expressed in raising the intensity of bioenergetic processes, in the activation of detoxication systems and the biosynthetic regenerative processes. The multi-factor mechanisms of ozone curative effects in case of wound dystrophy and burn disease are based on the direct ozone reaction with organic substrates (carbohydrates, proteins and lipids) and its indirect impact on homeostasis due to the generation of ozonides (products of the interaction of ozone with fatty acids and lipoproteins). The ozonation of those different substrates at physiological pH level results in the formation of a complex and heterogeneous pathways of components, and the modulation of bioactive products synthesis. Ozonides provide to the organism some prolonged reserve of active oxygen to maintain the aerobic metabolism and the required level of energetic substrates. In the same way the monooxygenase system activity is simulated directly in a vascular bed. High rates of the ozone interaction with amino and fatty acids facilitate the more balanced development of compensatory-adaptive reactions of the organism in case of pathology.[1-5]

The local oxygen-ozone therapy for wounds and superficial scalds results in accelerated cleansing of a wound from necrotic tissues without originate necrosis. When applied for deep burns it causes the necrotic patch lysis without using enzyme preparations; ensures the

retention of vitalized tissues of a paranecrotic zone, the quick management of pain and edema, the reduction of epithelialization time and a better graft retention.

The systemic ozone therapy induce the oxygen homeostasis reactivation, increase antioxidant activity of blood plasma, improve the peripheral circulation and microcirculation, make possible the homeostasis compensation and detoxification, and modulate the inflammatory and immune respond.

Ozone Therapy in Traumatology

During the elaboration of the methodology for the local application of ozone in patients with chronic osteomyelitis A.B. Zaytsev (1998)6 suggested the local open bone and soft-tissue wound treatment with an ozone-oxygen mixture at a concentration of 1- 5 mg/L within 20 min. Applied this procedure, wound of 7 patients from 16 ones healed by secondary adhesion within a mean wound healing time of 28.1 ±10.1 d. Wounds of 9 patients were prepared for autodermoplasty within 13.3±4.2 d. The qualitative and quantitative testing of wound discharge showed the statistically decrease in the number of pathogens from 360 to 30 CFU/cm ($p<0.02$) on a wound surface as compared to patients treated with the conventional method, whose wound bacterial content level was reduced only from 436 to 172 ($p<0.02$) CFU/cm. The analysis of the wound cultures composition showed the reduction of the gram-negative flora by three times just after the first procedure with its further complete disappearance within 7-10 d. At the same time there were observed the reduction of associate's microbe and an improvement of the antibacterial sensibilities of *Staphylococcus aureus and Streptococcus haemolyticus* to antibiotics.

The clinical analysis of patients cured using local ozone therapy shown the activation of the regenerative process. The cytogram of wounds during the ozone therapy was characterized by signs of purification as follows: the number of neutrophils in the exudate was reduced within 5-7 d, lymphocytes, macrophages and neutrophils appeared with signs of complete phagocytosis. During the second week the number of lymphocytes and monocytes decreased, macrophages began to prevail and the fibroblasts appeared.

In N.V. Zhigalenkova's investigations (2000)[7], there were elaborated the clinical protocols for using immunomodulators (Myelopidum, Imunofan), an ozone - oxygen mixture and a metabolic therapy in the system of complex pathogenetic-reasonable treatment of purulo-septic complications in trauma - orthopedic patients. Optimal conditions were determined for the ozone therapy inclusion in the integrative treatment of proinflammatory processes of the supporting-motor system for the stimulation of the functional activity of local immunity factors. The ozone therapy was used in the integrative treatment of 65 patients with purulent processes and complications of the supporting-motor system. The detailed immunoassay was carried out in 39 of them. There were 25 patients with posttraumatic osteomyelitis, 11 patients with postsurgical and 3 patients with gunshot one.

The following rational scheme was suggested for using the ozone therapy: once a week, 2-3 intravenous drop-by-drop administrations, at dose of 4-5 mg ozone per 1 liter in 0.9 % NaCl

normal saline solution, in a volume of 200- 400 mL. It was determined that the ozone therapy should be carried out in combination with the surgical treatment and the prescription of drugs stimulating the cellular immunity (Myelopidum, Imunofan).

The immunoassay data analysis for patients examined before and after the ozone therapy showed a considerable increase of neutrophil activity in both spontaneous and stimulated luminol-dependent chemiluminescence compared to initially reduced level. After the ozone therapy a considerable increase in the formation of reactive oxygen species was noted in patient compared to the initially normal level of chemiluminescence. An increase in phagocytosis was revealed in a group of patients with active (acute) suppurate process and with slow evolution.

According to clinical data the general health status of a considerable part of patients improved after the ozone therapy. Patients express their higher vivacity and improvement in appetite, increased working ability and other live qualities indices. In the course of the dynamic monitoring of patients their intoxication indices was reduced, the pain syndrome decreased and the body temperature was normalized. During bandaging a purulent fistulous discharge was reduced as well as inflammatory and local irritation. Neither adverse nor allergic reactions were observed after the ozone therapy. It was noted that in patients with allergic or auto immune diseases, such ozone therapy should be carried out with care, after the allergist and rheumatologist's consultation. In two of 39 patients treated with ozone therapy the exacerbation of purulent process was noted after their remission from hospital. Immunodeficiency in those patients was observed (low content of T- and B- lymphocytes). No immune correction was performed in those patients, so it might result in an acute condition of purulent process.

L.S. Vegele's research (2003)8 included the study of the combined effect of ozone and Poviargolum (silver containing medicine) on the septic wound microflora of trauma-orthopedic patients with suppurative complications. The study involves the analysis of indicators of changed responsiveness (phagocytosis, circulating immune complexes) and lipid peroxidation. In this context was analyzed the effectiveness of this integrative therapy. It was determined that the combined use of the local ozone therapy with silver containing drugs in low concentrations promote the bactericidal activity and the increase in responsiveness to polyantibiotic-resistant microflora to antibiotics, in addition facilitates the fast debridement of hard-to-heal septic wounds.

The ozone therapy was applied under the following scheme: the infected wound surface of 96 patients was treated after the removal the bandage with ozonized water by washing purulent discharge from the wound. All devitalized and necrotic tissues were removed mechanically. The open wound was further irrigated with an ozone-oxygen mixture of the 500 μg/L concentration within 20-30 min in a chamber for the external ozone therapy. After that a septic wound was treated with 1% sodium bicarbonate solution to neutralize the medium. Then a 1% Poviargolum solution was applied.

Such management of a wound was performed daily during bandaging till the complete epithelialization of the wound or till the complete debridement of the wound surface and its preparation for skin grafting. On the 2^{nd}-5^{th} day of the beginning of the therapy the purulent discharge was reduced and the gradual wound cleansing from necrotic particles occurred. At

the same time new granulations tissues emerged and the gradual epithelialization began. It was noted an improvement in all the patients subjected to this treatment. The majority of them stated with a reduction in the pain syndrome. Neither complications nor adverse effects were observed.

During the treatment with ozone and Poviargolum used in the both cases in low concentration there were noted the recovery of the antibiotic sensitivity of microflora discharged from septic wounds, i.e. the antibiotic spectrum was enhanced. The analysis of the data showed that the extension of the antibiotic sensitivity spectrum was more evident in gram-positive microorganisms, in particular in groups of antibiotics suppressing the microorganisms' protein synthesis, such as: aminoglycosides, macrolides, lincosamides and chloramphenicol. For example, the group of aminoglycosides has a selective effect on the nucleic acid exchange, specifically by suppressing the RNA synthesis. Besides, the spectrum of sensitivity was extended to beta-lactam antibiotics, belong to the penicillin and cephalosporin group, inhibiting the synthesis of microorganism's cell wall.

The main increment in the antibiotic sensibilities of gram-negative bacteria was reached in the group of aminoglycosides, chloramphenicol and polymyxin. The extension of the spectrum of sensitivity to those antibiotics after the treatment of the wounds can be explained by the changes occurring in a cell wall of microorganisms under the exposure to ozone. Ozone can induce the deterioration of microorganism's cell wall permeability or suppress protein and amino acids synthesis resulted from the addition of Povyargolum. Ozone can facilitated the penetration of the bactericidal component of Povyargolum (Ag^+) inside microorganisms and accelerate their destruction.

As a result of the combined treatment the inflammation in all 96 patients (100% cases) was reduced, wounds were completely cleansed from pathogen microflora, confirmed bacteriologically. The number of circulating immune complexes (CIC) was considerably reduced ($P<0.05$). A tendency appeared for increasing phagocytosis indices. The total wound epithelialization occurred in 78 (81.25%) patients. For the rest 18 (18.75%) patients during the total debridement of wounds in 15 (15.6%) cases skin grafting was performed, in three (3.1%) cases secondary stitches were put.

In patients treated with conventional method (standard antiseptic protocol), the total wound epithelialization take place in 7 (35%) patients within longer periods of recovery. Skin grafting was performed to 4 (20%) patients. The rest 9 subjects (or 45%) were derived to be treated with other treatment. The mean area of the wound surface of those patients was 13.55±6.36 $cm^{2.}$

The combined application of two antiseptics Ozone + Povyargolum used in low concentrations has enabled to obtain a synergic effect together with better regenerative processes in wound healing. As a result of the local treatment of patients with hard-to-heal purulent wounds performed by the suggested integrative medication (using low-concentrated drugs) the total epithelialization was achieved in 81.25% cases and the rate of the relative reduction of a wound area just at the beginning of treatment came to 8.84%.

Ozone therapy in burns

The guidelines for high-tech medical care delivery to severe-burnt patients were based on active surgical procedures including early necroctomy, the accelerated preparation of wounds to grafting, autodermoplasty, early reparative-plastic surgery, the application of up-to-date wound coatings, the skin cell grafting cultured, intensive infusion-transfusion therapy, efferent therapy, diagnostic-and-treatment fiber-optic bronchoscopy and fiber gastroduadenoscopy, enteral and nutritional support, multicomponent drug therapy, surgical and conservative methods of after-burn treatment. The methods of the local and systemic ozone therapy may be used in most of the described components as integrative treatment in heavy-burnt patients.

Local Ozone therapy for heat injury

The primary scopes of the local ozone therapy in heat injury treatment are: the control of inflammation, the elimination of infection and the reduction of wound healing time. To afford those challengers ozone can be uses based on the bactericidal, virucide, fungicidal effect, its capability to stimulate tissue restoration and regenerative processes. In addition, ozone can improve the microcirculation in a wound surface region, to enhance the supply of a wound surface with oxygen. Integral local treatment of wounds with ozone ensures the elimination of the necrotic tissues within 5 days without necroctomy. This procedures guaranty the complete wound cleansing in deep burns of 3rd-4th degree after necroctomy, without enzymatic treatment. In addition, after that, the throphism of the tissues improve and the viability of tissue in a pranecrotic area increase, as a consequence is reached a faster reduction of a wounds area.

During the design of the guide lines for the local ozone therapy application in burn wounds A.A. Struchkov (2007)9 showed that the selection of the clinical procedures depends on the injury depth, the stage of the wound process, the burn disease course and the wound localization. The burn treatment should be integrated including the surgical treatment of wounds and an adequate infusion-transfusion therapy, the use of antibiotics and other drugs preparations.

Treatment of wounds with an oxygen-ozone mixture in a plastic bag. Such method is applicable in case of injury in limbs. The method consists on: A moistened bandage with saline solution or distilled water is applied on an affected skin area. A plastic bag (chamber) is put onto an extremity and the wound irrigation with ozone is performed therein.

The treatment of superficial non-infected scalds, the scope is to maintain a low bacterial contamination of wounds in order to facilitate the self-healing stimulation. For this purpose it is sufficient to carry out daily treatment with ozone at 1.5- 2.0 mg/L at gas flow speed of 0.5 – 1.0 L/min and the exposure time 25-30 min. Procedures shall be carried out twice or three times a day. A wound scab should not be thicker than 2 gauze strata and should not be removed unless necessary. The general requirements shall be following: pre-drying of scab with preheated air.

In treatment of deep burns by using ozone the following three main problems are present: I. Wound healing. II. Obtaining better results of autodermoplasty. III. The prevention and treatment of wound healing and hard-to-heal wounds, decubital sores.

I. Wound preparation for the surgical restoration. The first step after necroctomy is to suppress the pathogen microflora and to stop the inflammatory reaction. For this purpose the irrigation is performed in a plastic bag with the ozone concentration of 5-10 mg/L at gas flow speed of 1 L/min and the exposure time 20-30 min. Procedures shall be carried out once or twice a day. The irrigation is supplemented with subcutaneous injection of ozone along the perimeter of the wounds at a distance of 2 cm from the wound edge. The ozone concentration in such injection mixture is 2-3 mg/L, 3 - 5 mL of the mixture is to be administered subcutaneously or intramuscular at injection intervals of 7-10 cm daily or every other day.

The wound cleaned and bandaging by using an ozone-containing normal saline solution with the ozone concentration of 2 - 4 mg/L therein shall be carried out daily until the complete healing. The ozone concentration shall remain the same in the solution till the disappearance of inflammation signs, the cleansing of a wound from purulo-necrotic substance and the occurrence of a granulation tissue in the wound. Further on, the concentration stimulating the tissue reparation (0.5-1.0 mg/L) is used for wound healing. The application shall be repeated 10-20 times. In case of wound healing by the second intention after its cleansing, ozonized vegetable-oil (5-10 application) may be used to stimulate tissue reparatory processes.

For injured persons, to whom a flow-aspiration drainage is indicated (mainly with the deep localization of a suppurative focus), an ozone-containing saline solution is effectively used as a perfusion medium in a volume of up to 400 mL per day (saturating saline solution at ozone concentration up to 10 mg/L). A drainage system is blown through 2-3 times a day by an oxygen-ozone mixture within 10-15 min at the ozone concentration of 50 mg/L. The ozone therapy is applied till the inflammation disappearance and the lack of microbial growth signs in lavage fluid samples became evident. A wound is further healed under aseptic condition.

In case of hard-to-heal wounds, smoldering wound process with the considerable quantity of thick wound discharge, the method of vulnerosorption with ozone is to be used. This method involves an absorbent agent treated with ozonized sodium chloride solution at the ozone concentration of 5 mg/L. In addition to the curative effect of absorbents, ozone potentiates the beneficial effect. Vulnerosorption is also carried out into a chamber using the compression-vacuum mode of ozonation. Hard-relief wounds with purulent ulcers and leakages are managed in baths with ozonized water or cleansed by jet of ozonized water with the use of a hydraulic pump device. The use of the ozone therapy as a combined method in case of wound infection reduces the time of treatment and decreases the use of drugs.

II. To improve the graft viability and to prevent infection within the first five days after autografting, the ozone therapy is to be performed in plastic bags with the ozone concentration of 1-2 mg/L at the gas flow rate of 1 L/min at the exposure time 25-30 min.

III. In the treatment of patients within the first two months after the skin integument restoration at the wounds healing stage it is recommended to apply hydropressive massage with ozonized distilled water. It is carried out by circular spray application at a rate of 5 s/cm^2. The ozone concentration at the device outlet will be 3-5 mg/L. To ozonize 1 L of water, the bubbling time should be not less than 40 min. The use of ozone by the above methods enables to improve the skin autograft retention and to reduce the time of hospital treatment of injured persons.

The ozone-gassing method under reduced pressure was first indicated for curing ulcers caused by radiation and hard-to-heal wounds. The main protocol of low pressure gassing consists on: The O2/O3 mixture is supplied from an ozonator into a synthetic cap, covering the area to be treated and is pumped under the controlled reduced pressure. As hemorrhage or wound discharge appears sometimes during gassing, an appropriate cuvette is connected to the system. Procedures are carried out under the pressure of -0.1 – 0.3 atm at the flow rate of 1-3 L/min with the ozone concentration of 2-5-20-80 μg/mL. The reduced pressure is continuously maintained so that the cap should bear tightly against a surface to be treated, should not suck in atmospheric air. The exposition takes from 10 min to 20 min.

Subcutaneous injections are given with the O_3/O_2 mixture at the concentration of 1 mg/L to 5 mg/L, 2-5 mL of the gas mixture is used per site of injection. The affected area is injected in a circumferential direction at a distance of 0.5-2.0 cm from the hyperemia boundary. Medical ozone may be injected daily or every other day. The treatment time depends on the clinical evolution of the pathological process.

Intramuscular injection: Most often the application of this method in burn patients is used when a non-clostridial or anaerobic infection is present in a wound. When revealing a similar infectious in an injured person, after the broad debridement of the skin and fascia incisions, wounds is irrigated with ozonized saline solution at the ozone concentration of 4–5 mg/L or administered using tissues microirrigators with an oxygen-ozone mixture (at a concentration of 10-20 mg/L, volume of 10-20 mL). The gas mixture is injected slowly in order to avoid painful reaction.

By combining ozone application methods it is possible to optimize the course of a wound process at any stage. However the adhere to the following key aspects should be recommended: 1) Test methods to the follow-up of the lesion evolution: microbiological cultures, cytomorphologic, planimetry and the determination of pro- and antioxidant balance in blood. In some cases it is convenient to add the study of microcirculation, the heat-scanner examination, the gas balance measurement in an arterial and venous circulatory bed. 2) The use of the ozone therapy in case of heat injury should be integrative – i.e. to combine methods of local and systemic action.

During the preparation of wounds for autodermoplasty, depending on the state of wounds, two radically different approaches are possible – one is mainly aimed at the debridement of wounds, the second one is focus to their regeneration. In case of profuse purulent discharge the irrigation with the ozone-oxygen mixture is performed at a concentration of 5-10-20 mg/L, the exposition time will be 25-30 min, at gas flow rate of 1L per min, once or twice a day, combined with ozonized saline solution, baths with ozonized solution, hydropressive wound treatment with ozonized solutions, irrigation with ozonized absorbents. As soon as a wound is cleansed and dried, the irrigation will be daily performed in plastic bags using ozone-oxygen mixture at the ozone concentration of 2-3 mg/L, for 25 min.

In case of autodermoplasty the main objective is to restrain infection and to preserve transplants. Therefore, ozone concentrations will not exceeding 2 mg/L and will be applied daily during 25 min. After the implant, stem grafting the most important task is the restoration of the normal circulation in the stem and the development of a vascular tree in the transplanted tissue.

To stimulate reparatory processes, subcutaneous injections into base of the stem are made with the ozone-oxygen mixture at 2.5-3.0 mg/L, 3 - 5 mL per site of injection.

At burn units of hospitals the bactericidal effect of ozone is mainly aimed at the superinfection and reinfection of burn wounds because the microflora is destructed during the first one or two ozone-oxygen therapeutic procedures. The clinical implications of the bactericidal effect of ozone are: a smooth course of a wound process, the quick elimination of inflammatory process and signs of ascending infection in case of treatment of infected burns.

Ozone as **Necrolytic Factor**. The experience in treatment of wounds of different origin with necrotic tissues, enables to distinguish and describe one of the main effects of ozone-oxygen mixtures, which is called necrolytic one. The ozone property to destroy dead tissues leads the quick wound cleansing and thus furthers its earlier healing. Such necrolytic action of ozone was most evident in patients with superficial scalds.

A thin layer of liquefactive necrotic tissues was turned layer-by-layer during the treatment into tissue detritis. The necrolytic effect was evident under the ozone concentration of 9.0 mg/L and it grew with its strengthening. The cleansing of wounds with superficial scalds began on the 4th day and completed by the 9-10th day (in a control group it began on the 7th and completed by 15.5th days). The necrolytic action of ozone was only noted on liquefactive necrosis and it required the application of ointments with low osmotic properties. In case of deep burns the necrolytic effect became evident after escharectomy by melting devitalized tissues without using enzymatic drugs. The total wound cleansing from necrosis ended by 16.5 days (in a control group it was by the 19th day).

Ozone as **Trophic Factor**. The ozone-oxygen mixture causes a pronounced effect on living tissues of burn wounds. It may be considered as a trophic action when summing up all effects, which revitalize the viable tissues and further induce an accelerated regeneration. The clinical evidence of such trophic effect appear in patients with burns like bright hyperemia of a burn or granulating wound, by emerging dilated capillaries in ischaemic zones in the form of red dots, by fast pain relief and extremity edema reduction, by the skin sensitivity recovery, by active epithelialization, by the growth and maturation of granulations. Those clinical actions of all therapeutic factors on a burn wound result in a smooth course of a wound process. The wound cleansing begins and ends quicker and the tissue regeneration passes actively as far as a wound cleansed, thus, reducing considerably the time of treatment.

The mechanisms of the O_2/O_3 mixture on burn wounds were supported by the cytological examinations. There were analyzed 86 cytograms of 22 patients exposed to the ozone-oxygen therapy and 78 cytograms of 20 patients in a control group. The lack of any signs of a pronounced purulent inflammation in wounds were confirmed by cytogram data: the faster decreasing number of cells and their destruction degree in wound discharge imprints as compared to the control group, the considerable reduction of leukocyte quantity (by the 9-11th days), single or small accumulations of microbial cells throughout the course of treatment. As distinct from the control group on the 9th-11th day microorganisms were mainly revealed intracellularly, thus testifying active phagocytosis.

As a proof of such trophic impact stimulating regeneration processes was an unusually early emergence of fibroblasts alongside with macrophages in a wound discharge, which were detected from the 4-7th days as single and by the 12-14th days were met as groups in preparations, epithelium and fine connective-tissue fibers were also revealed therein, in contrast with the evolution of the control group.

The most important was that during the whole period of the ozone-oxygen therapy the correlation of immune competent cells such as heterophilic leukocytes, macrophages and lymphocytes was revealed in cytograms. In addition, there was evident an active local cellular immunity as compared to the control group, where within 2 weeks the inflammatory type of cytograms remained with prevailing heterophilic leukocytes, macrophages occurred rarely and no fibroblasts responsible for the regeneration were detected.

If the therapy course was started quite late after burn injury (after 20 days) with a complicated course of burn wounds, when initial cytograms had an inflammatory type, it was detected that after 2-3 treatment cytograms turned to inflammatory-regenerator type and then to regenerator one with the reduction of the total number of cells and the percentage of their destruction, while the number of macrophages and fibroblasts increased and the number of a heterophilic leukocytes decreased. Those signs demonstrated the germicidal and trophic effect of the ozone-oxygen mixture on a burn wound with a complicated course.

Local ozone-oxygen therapy should be appropriated in those conditions: 1) Superficial and deep burns at early stages. 2) In complicated course of burn wounds (at any stages after a burn injury): pronounced purulent inflammation, signs of ascending infection, slow cleansing process and retarded regeneration. 3) Burns in patients with burdened anamnesis (atherosclerosis, obliterating endarteritis, diabetes mellitus, multiple drug allergy).

Contra indications: In patients with general grave condition.

Recommended Treatment Protocol: For superficial burns at the early beginning of treatment with a non-complicated wound process low ozone concentrations are indicated for the whole course of treatment. In cases of high initial microbial invasion the treatment in the destruction phase is to be started from 10-20 mg/L. To proceed under a non-complicated state, to lower concentrations may be used. At late stages after arresting evident signs of inflammation, the treatment course should consist of 10-15 applications.

For deep burns with minor initial microbial invasion should be started the treatment with low ozone doses in the destruction phase and mean ozone concentrations are used in the inflammation phase and continued till the time of autodermoplasty. In cases of high initial microbial invasion the treatment is started from mean ozone doses in the destruction phase with their retaining under non-complicated course till operation. In case of a complicated course the treatment is also started from high (or mean) ozone concentrations. The local therapy course should consist of 12-15 applications.

The systemic use of the ozone therapy in burns treatment

The method for the systemic use of ozone in the course of the burn disease restorative treatment includes the set of parenteral methods of administration of oxygen-ozone mixtures in the form of an ozonized saline solution or ozonized autohemotherapy; ozonized components of blood having an effect on different systems and organs.

The necessary supplement to the suggested treatment schemes is the dynamic control of their effectiveness based on the study of the state of pro- and antioxidant balance of patient's biological fluids and the microcirculation state by using the Doppler sonography laser. The application of the recommended technology enables to expand considerably the range of the systemic ozone therapy use and to increase the degree of its effectiveness.

The indications for the systemic therapy are superficial and deep burns on an affected area of over 5% of the body surface, burn disease and wound dystrophy, complications of burn disease and wound dystrophy on the part of cardiovascular, respiratory, nervous systems, gastrointestinal tract, liver or kidneys.

The systemic use of the ozone therapy in acute pathology is often associated with difficulty to estimate the required dose of active oxygen in conditions of developing oxidative stress, the pattern of available deficit of antioxidant reserve unknown for an ozone therapist. Therefore, an individual adjustment of a dose to a patient is first of all necessary for acute clinical pathology.

Ozone dose individualization

Prior to prescribing the parenteral ozone therapy or drugs with antioxidant properties 20 mL of blood will be taken from a patient using heparin as anticoagulant to assess the patient's the oxidative status. The lipid peroxidation (LPO) intensity and the state of the antioxidant protection system in blood plasma will be measured by the induced biochemicoluminescence method.[10]

At the next stage of the study an ozone dose is determined, which may cause oxidative stress (the lipid peroxidation intensity increases and antioxidation activity decreases according to the biochemicoluminescence data). For this purpose blood is divided into a required number of aliquots corresponding to a number of ozonized saline solution concentrations (ozone doses) intended for administration. Then an ozonized saline solution is sequentially added into blood samples with different doses of ozone in a volume proportional to the ratio of an infused solution and a circulating blood volume (for example, 0.1 mL, 2 mL respectively), into a control blood sample non-ozonized, sodium chloride in place of the same is added, to obtain the hemodilution level. Blood samples are exposed within 10 min and centrifuged at 1500-2000 $r \bullet m^{-1}$ by 10 min. Blood plasma obtained is used for the oxidative status assessment by using the induced biochemicoluminescence. When indicating a course of the intravenous ozone therapy to a patient such dose of ozone is selected, therewith in conditions *in vitro* the moderate initiation of oxidative processes occurs with prevailing antioxidant protection system. In case of need to prescribe antioxidant preparation for further investigation such dose of ozone is selected, therewith in *in vitro* conditions the oxidative stress occurred (the lipid peroxidation activation

and the antioxidant protection system depression).

Remaining blood is divided then to equal number of samples corresponding to the number of preparations tested. At first to blood samples a saline solution is added with a defined ozone concentration, which has caused the oxidative stress. After this, different preparations are sequentially added in doses corresponding to the blood volume. Blood samples are exposure by 10 min. To the patient is prescribed a preparation, which has demonstrated in conditions in vitro the highest antioxidant activity (the LPO/AOA ratio index should decrease as compared to a non-treated sample, wherein oxidative stress has occurred under the effect of the ozonized saline solution).

Results of the parenteral ozone therapy in case of burn disease

The clinical studies of the effective parenteral ozone therapy (the intravenous administration of the ozonized saline solution) were studied as a part of the integrative infusion-transfusion and drug therapy in 184 patients with heat injury. [5,9] The main group was represented by 112 patients aged from 23 to 64 years with burns of the II-III-IV degrees and a burning area from 15% to 64 % of the body surface, who were prescribed as a part of the integrative treatment a course of ozone therapy. Ozone was supplied under control of pro- and antioxidant systems by the biochemicoluminescence methods with individual dosage of an administered pro-oxidant. In a control group (72 patients aged from 26 to 71 years with burns of the II -III - IV degrees on an area from 17% to 58 % of the body surface) the standard protocol infusion-transfusion and drug therapy was carried out).

Indications for the prescription of the parenteral ozone therapy were: clinical signs of hypoxia (increased lactate level, reduced lactate dehydrogenase activity, hypoxemia), toxemia, i.e. the availability of endotoxicosis (increased levels of glucose, createnine, urea, the concentration of medium molecules, fibrinogen degradation products, LPO intensification); disorders in the cardiovascular system (the hypodynamic state of cardiohemodynamics, microcirculation disorder), the external respiration malfunction.

At the start point in most of the patients at early stages of burn diseases, changes in pro- and antioxidant systems were detected. Changes were characterized by the oxidative stress, mainly the increased LPO, the accumulation of primary, intermediary and final lipid peroxidation products were observed with the simultaneous reduction of the antioxidant system activity. Changes of the metabolic homeostasis in patients' blood during the developed hypoproteinemia and hypoalbunemia were characterized by increasing indices of endotoxicosis (increased levels of glucose, createnine, medium molecules and hyperenzymemia).

At the stage of acute burn toxemia when the primary task is to reduce toxins in blood, to restore microcirculation and to prevent complications in internal organs (such as acute gastric ulcer), the intravenous administration of ozonized saline solution (200-400 mL) may be used with the ozone concentration of 3-5 mg/L at the output of the apparatus. In critical cases against high intoxication the major autohemo ozone therapy is carried out with a single dose of 500 µg by plasmapheresis. To afford signs of incipient enteroparesis or at increased risk of the gastric

ulcer development, gastrointestinal dialysis is performed with ozonized water.

At the septicotoxemia stage the problem of the septic state suppression, burn anemia prevention, immune disorders control is advanced to the foreground. In this period such systemic action may be used in the treatment as the administration of an ozonized saline solution for ozonized autoblood, erythromass and rectal insufflation of ozone-oxygen mixture.

Patients with burn disease at the stage of septicotoxemia (59), aged from 34 to 67 years were examined. They suffering from burns of the 3rd-4th degrees on an area of 24 -48 % of the body surface.5,9 Methodology: during two weeks after the transfer of patients from an intensive care unit (ICU) 30 patients were subjected to the standard complex infusion-transfusion and drug therapy (control). To a group of 29 patients (experiment) the parenteral ozone therapy was applied by daily infusion of the ozonized saline solution (120 -240 μg O3), autohemo therapy with O3 (300 - 500 μg) under control of blood chemiluminescence analysis twice or three times a week.

The central and system hemodynamics were studied using the tetrapolar rheography method. The tissue mircocirculation was appraised by using the computer laser Doppler fluometry (analyzer ЛАКК-01, RPE LAZMA). The following indices of the clinic-biochemical status were studied: pro- and antioxidant equilibrium by the chemiluminescence method; acid-alkali balance of blood; blood-clotting factors; lactate, glucose, middle molecules, urea contents; aspartate- and alanine amino transferase activity, proteolytic enzymes, contents of protein and protein fractions.

The parenteral ozone administration was resulted in the elevation of a pro-oxidant potential in burn persons. The chemiluminescence activity increased by 8–12% from the initial level. Against that background an antioxidant potential increased greater, by 25–28% of the initial indices level. Such nature of changes in LPO and antioxidant protection indices was the most optimal and accompanied with positive changes in other oxygen-dependent parameters of the organism homeostasis.

The study of the systemic and central hemodynamics during the parenteral ozone therapy proved its positive effect on the metabolic status of the myocardium and the recovery of its functional capabilities. The most typical were the dynamics of changes in the heart rate and cardiac output: as compared to the control group, the stroke volume growth was more pronounced at a lower heart rate. The capillary blood flow parameter dynamics was analyzed by the laser Dopplerography method (LDF) of the perifocal zone of burn wounds after systemic ozone therapy procedures and courses.

Data obtained on the capillary circulation state showed that at once after a systemic ozone therapy procedure and further on during the systemic ozone therapy an amendment of perfusion in a near-wound zone were noted. It was marked in a more significant increase of microcirculation index in a test zone as compared to a control area. And herewith active mechanisms of the blood flow regulation prevail over passive ones. The similar recovery of the peripheral circulation and microcirculation was generally typical for the whole organism. It played an essential role in the provision of compensatory-adaptive reactions of the organism

in case of burn disease.

One of the proofs of the positive effect of the systemic ozone therapy in early period of burn disease was the proteome recovery dynamics. The protein potential recovered under the action of the parental ozone therapy more effectively than under the standard therapy. After the parental ozone therapy the general state of health grew better in most of patients. It was proved by general feeling of well-being, better appetite and higher work capacity. Under dynamic monitoring in patients the intoxication reduced, the pain syndrome decreased and the body temperature was normalized. Thus, the positive clinical effect was obtained by using the ozone therapy in the integrative treatment of burn disease.

Possible complications

Possible complications of the method include allergic reactions, which occur as itch, rash, burning sensation, breathing difficulty etc. In case of onset of the said symptoms it is necessary to interrupt the treatment, to prescribe antiallergic therapy (hormonal preparations, antihistamines etc.). One of the complications may be the development of phlebitis. Phlebitis along the veins results from using high (over 4-5 mg/L) ozone concentrations saturating saline solutions and frequent infusions of such solutions into the same peripheral vein. Besides, phlebitis may develop after re-transfusions of ozonized autohemo therapy with high ozone doses (2000-3000 μg) and into the peripheral vein.

Such complications may be prevented by the reduction of the ozone dose when using both ozonized saline solution or autohemo. In addition, the systemic ozone therapy must be carried out under control of the functional state of pro- and antioxidant systems by the biochemiluminescence analysis method.

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