



**XI RUSSIAN SCIENTIFIC CONFERENCE IN
OZONE-THERAPY
VI INTERNATIONAL JOINT CONFERENCE
RUSSIAN-IMEOF-AEPRIMO**

**Ozone: active forms of oxygen, nitric oxide
and high-intensity physical factors in biology and medicine.**

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Abstract Book

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XI RUSSIAN SCIENTIFIC CONFERENCE IN OZONE-THERAPY

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Abstract Book

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NITRIC OXIDE AND LONG-TERM SENSITIZATION IN TERRESTRIAL SNAIL

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Long-term sensitization (LTS) is a form of a long-term plasticity, in which there are signs of both nonassociative and associative learning. Previously, we found that the production of LTS in terrestrial snails depends on calcium ions; it was shown that LTS is accompanied by a decrease in the membrane and threshold potentials of the command neurons of the defensive reflex. Nitric oxide (NO) is considered in present as a new signaling molecule, which plays the role of a universal regulator. There is a lot of evidences of its involvement in plasticity-related processes. Therefore, the aim of this investigation was to study the dynamics of NO products during elaboration of LTS in the terrestrial snail.

The terrestrial snail *Helix lucorum* was chosen as an object for experiments. LTS of defensive reflex closure of the pneumostome was received by application of electrical stimuli in the head 4 times a day with intervals of 1.5-2 hours for 4 days. We studied the change in the production of NO during elaboration of LTS using the electron paramagnetic resonance (EPR) spectroscopy method. EPR measurements were carried out in the X-range spectrometer EPR ER-200 of Bruker company at a temperature of 77 K. Typical EPR spectra of nervous system and heart tissues represent triplet signals from the spin trap complex with iron and nitrogen oxide. The intensity and area of these signals is a measure of the amount of NO that is produced for the time being the spin trap in the animal's body. The obtained results show that after the elaboration of LTS of the defensive reflex, the intensity of NO production in the examined tissues of the snail decreases.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University (No 17.9783.2017/8.9) and Russian Foundation for Basic Research (grant 18-015-00274_a).

Keywords: nitric oxide, nerve system, long-term sensitization

CONDITION OF FREE RADICAL OXIDATION DEPENDING ON BODY FAT MASS (ACCORDING TO BIOIMPEDANSOMETRY) IN ADOLESCENTS WITH NORMAL BODY MASS INDEX

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It is interesting from a practical point of view to determine the role of excess body fat mass at normal BMI indicators in the risk of metabolic disorders, including oxidative stress. **The aim of the study** was to evaluate the characteristics of free radical oxidation in adolescents with a normal body mass index (BMI), depending on body composition according to bioimpedansometry.

Materials and methods of research

The main group included 75 children of adolescence (11-17 years old) with normal parameters of BMI (25th percentile $< \text{BMI (kg / m}^2) >$ 75th percentile) of the 1 st-2 nd group of health. Then all adolescents of the main group were divided into 2 subgroups depending on the % BFM index according to bioimpedansometry. The 1st group consisted of 30 adolescents with % BFM indicating excess weight or obesity. The 2nd group included 45 adolescents with % BFM corresponding to the norm or fitness standard. The comparison group was presented by 30 adolescents with primary uncomplicated obesity of the 1st-2nd degree (according to the body mass index).

All the adolescents were tested for anthropometric indices (body mass index, waist circumference), a general blood test, a biochemical blood test with determination of lipid parameters (total cholesterol, triglycerides) and carbohydrate (glucose) metabolism, fibrinogen. An oral glucose-tolerant test, daily monitoring of arterial pressure were held. Bioimpedansometry was used to evaluate the composition of the body. It was held on the apparatus of Medass (Russia). Evaluation of parameters of free radical oxidation (FRO) was carried out by the method of induced chemiluminescence (IChL) of blood serum on the domestic biochemiluminometer BLM 3606 M-01 (Russia). The value of the total light-sum (S total) and the first peak of induced chemiluminescence (H (conditional units)) were determined. In addition, an integrated indicator - chemiluminescent oxidation coefficient (ChOC): $(S \text{ total} \times H) / 10^6$ (unit units) was calculated in the examined children. It indicated the state of the oxidative status of the child as a whole. STATISTICA 6.0, Microsoft Excel 2003 were used for statistical processing of the material.

Results and discussion

A comparative analysis of parameters of oxidative status in the examined groups showed that in adolescents of the 1st subgroup H, S total ($p < 0,05$) and ChOC ($p < 0,05$) were higher by 17,7%, 21,0% and 42,5% than in the adolescents of the 2nd subgroup, respectively. At the same time, all the parameters of the oxidative

status in adolescents of the 2nd subgroup were significantly lower than the similar in the adolescents of the comparison group. And in children of the 1st subgroup only ChOC had a significantly lower value than the ChOC in the adolescents of the comparison group.

A further correlation analysis between anthropometry (BMI) and body composition (% BFM from bioimpedansometry data), on the one hand, and free radical oxidation of blood serum according to IChL (S total, ChOC), on the other, revealed various correlation links in the examined groups. So, a significant positive correlation between BMI and IChL parameters (S total ($r = 0,317$), ChOC ($r = 0,489$)) was recorded in adolescents of the comparison group. In adolescents of the 1st subgroup, a weaker positive correlation was recorded only between BMI and ChOC parameters ($r = 0,302$). A reliable positive correlation was also recorded between % BFM and IChL parameters in both the adolescents of the comparison group (S total ($r = 0,408$), ChOC ($r = 0,562$)), and in adolescents of the 1st subgroup (S total ($r = 0,364$), ChOC ($r = 0,472$)). In adolescents of the 2nd subgroup, reliable correlations between anthropometry (BMI) and body composition (% BFM), on the one hand, and serum FRO parameters (S total, ChOC), on the other hand, were not revealed.

Conclusion

Features of the oxidative status in adolescents, depending on the composition of their body according to bioimpedansometry, indicate the role of excess % BFM in oxidative stress, which is one of the pathogenetic mechanisms of metabolic shifts at obesity. Adolescents with normal BMI, but overweight and obese according to bioimpedansometry, should be considered a high-risk group for the development of obesity and metabolic disorders.

Key words: oxidative stress, bioimpedansometry, body fat, children

ANTIOXIDANT STATUS OF THE ORGANISM AT COMBINED BURN INJURY TREATMENT OF COLD PLASMA

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Introduction

In the pathogenesis of the burn disorders development during the period of burn shock and toxemia, an increase in the activity of antioxidant enzymes (superoxide dismutase (SOD), catalase) is observed in erythrocytes due to the entry of decomposition products of damaged tissues into the blood. Therefore, the study of antioxidant link characteristics in blood at the thermal trauma and the search for ways to correct them is topical. The basis of used in the research the air-plasma scalpel-coagulator-stimulator SCAP / NO-01 "Plazon" is the effect on body tissues of the gas flow formed by cooling air plasma and containing NO molecules.

Materials and methods

Studies were carried out on 70 male Wistar rats weight in 200-250 g. Animals were divided into 7 groups: the 1st group is the intact animals, the 2^d, 3^d and 4th is control groups, the 5th, 6th and 7th is experienced groups. The rats of control and experimental groups simulated a combined thermal trauma (CTT) by applying a burned hot body to a 20% body surface (3c) and a thermo inflammation injury (10c); animals were deduced from the experiment on days 1st, 7th and 14th. Rats of 5th, 6th and 7th groups were irradiated daily by a stream of "Plazon" apparatus air plasma at the distance of 1 cm from the burn surface within 2 minutes. The concentration of nitric oxide reaching the burn surface was ~ 3000 ppm. In the studies, spectrophotometric methods used to determine the total activity of SOD in the 1:10 hemolysate, catalase in 1:100 hemolysate such as determine the protein content in the sample to calculate the specific activity of the enzymes. For the research, the Power Wave spectrophotometer (Japan) was used.

Results

The increase in the specific activity of SOD at the CTT on the 7th day was 3.29 times ($p = 0.031$) and on the 14th day it was 2.75 times ($p = 0.029$) respectively compared with the norm. Catalase activity increased 2.42 times ($p = 0.025$) on the 1st day, in 6.90 times ($p = 0.034$) on 14th day. The obtained data corresponds to the picture of developing hypoxia and the dynamics of indices in toxemia, ends between 7 and 14 days. We were get an increase in the specific activity of SOD on 2.20 times ($p = 0.033$) for 1st day and on 2.15 times ($p = 0.032$) on day 7, comparing the experiment with the norm. Also, an increase in catalase activity was found 3.13 times ($p = 0.035$) for the 1st day, 2.83 times ($p = 0.031$) on day 7 compared with the norm. The obtained data demonstrate the exit from the toxemia stage between 1 and 7 days. In comparison with the control

experiment, an increase in the specific activity of SOD was observed by 1.28 times ($p = 0.023$) on the 1st day of treatment, and on the 7th day decrease in 1.52 times ($p = 0.032$). Thereby, decrease of catalase activity was established in 1.64 times ($p = 0.027$) on the 14th day of treatment. Thus, we can assume that the use of "Plazon" in the treatment of thermal injuries can reduce the treatment time, increasing the healing rate of the wound, normalizing the antioxidant status.

Conclusion.

The statistically significant decrease was received in the specific activity of SOD at the 7th day, decrease in specific activity of catalase at the 14th day on 1.64 times. The data indicate an accelerated cure of thermal trauma with application of the "Plazon", being that a reduction in the stage of toxemia, accelerated normalization of the antioxidant status of the organism was established.

Key words: cold plasma, nitric oxide, burn injury, burn disease, superoxide dismutase, catalase

CAN BIOFILM BACTERIAL INFECTIONS BE TREATED WITH OZONE?

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One of the most common forms of bacteria is biofilms. By biofilm is understood: structured highly ordered community of bacterial cells, enclosed in a polymer matrix and attached to living and implanted in the body surfaces, having a system of internal communications and a system of interaction with the outside world. Being in the composition of biofilms, bacteria are protected from the action of phagocytes and antibacterial substances.

Ozone and ozonides can have a negative impact on all stages of biofilm formation.

Antiadhesive effect of ozone. Ozone has the ability to simultaneously attack the entire outer shell of the bacterium, including its functionally important formations: outgrowths, crypts, flagella, cilia, and being a bipolar molecule, it acts on electrostatic, hydrophobic, van der Waals processes and as a result of these interactions the repulsive force increases like surfaces and the primary contact of bacteria with the surface is disrupted.

The interaction of ozone with the signal molecules of the microbial community. In gram-negative bacteria, signaling molecules of interaction between microorganisms in biofilms are acylgoserine lactones. As a result of the interaction of ozone with acylgoserine lactones, they are oxidized, the lactone cycle is broken and a carboxylic acid is formed. Gram-positive bacteria perform communications in biofilms using oligopeptide signaling molecules. In the interaction of ozone with oligopeptides, the latter are oxidized to amino acids, as a result, the level of signal molecules of the microbial community decreases, which causes a violation of the exchange of information between microorganisms in the biofilm. The microbial community ceases to function as a single organism.

Disorganization by ozone of the matrix structure of the biofilm. Polysaccharide chains in biofilms are crosslinked by hydrogen bonds. Ozone, being a bipolar molecule, acts on intramolecular hydrogen bonds and, as a result, changes the spatial configuration of polysaccharides. The ozone-induced disorganization of the matrix structure of the biofilm opens up access to bacteria of antibiotics, phagocytes, ozone itself and ozonides and, as a result, allows eradication of the microbial population.

Key words: eradication, bacterial biofilm, ozone

NEW POSSIBILITIES IN THE TREATMENT OF COMPLICATED FORMS OF CERVICAL ECTOPY IN WOMEN WITH MISCARRIAGE

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Introduction

The main cause of high prevalence of urogenital infections, obstetric complications and spontaneous abortions is an increase in the period between the onset of sexual activity and marriage, as well as low awareness of the population about reproductive health.

Material and methods

There were 300 women under observation with a history of miscarriage and an ectopic cervix. To compare the effectiveness of the proposed methods of vaginal sanitation in preoperative preparation before the destructive treatment of the pathological focus on the cervix, in a simple, randomized way, all the patients were divided into groups that were comparable in their clinical characteristics. The first group consisted of 150 women who, before destructive treatment of the pathological focus on the cervix, were treated with medication, identical and mandatory for both sex partners, with the use of anti-inflammatory and antibacterial agents, the second group - patients (n = 150) , in addition to copper-kamentoznom treatment used ozonotherapy in the form of a small autohaemotherapy in both spouses. In addition, the vaginal instillation in women with an ozonized saline solution was combined with applications of the partner's urethra with ozonized olive oil Ozonid. For the entire period of treatment, patients were advised to use the barrier method of contraception (condom) in a constant regime.

Results

The combination of drug treatment and ozonotherapy made it possible to achieve almost complete elimination in women and men of the causative agents of the combined infection in 93.0% and 94.0% of observations, respectively, whereas with the use of traditional medicament therapy only, a positive effect was achieved in 65% and 62.0% of observations, respectively (p <0.05 in all cases).

Discussion

The basis for the action of ozonotherapy is the ability to reduce microbial invasion. The presence of infectious and inflammatory diseases of the male reproductive system leads to a decrease in the probability of conception of the child, the formation of a pathological embryo, an increased risk of complications of pregnancy and its spontaneous interruption. That is, it is the man who infects the woman with sexually

transmitted infections, which leads to a violation of the placenta microbioma and its incorrect formation, accompanied by a spontaneous termination of pregnancy in the early stages. Thus, the use of medical ozone in the framework of preoperative preparation before destructive treatment of a pathological focus on the cervix can improve the effectiveness of therapeutic measures, reduce the area of pathological changes, favorably affect the outcome of radio-wave destruction of the pathological focus on the cervix, reduce the epithelization time and the number recurrence of cervical disease in women.

Key words: cervical ectopia, miscarriage, medical ozone

THE INFLUENCE OF THE OZONE ON ACTIVITY OF ATPASE IN RATS ERYTHROCYTE UNDER EXPERIMENTAL BLOOD LOSS

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Introduction

The activity of adenosine phosphatase (ATPase) plays a leading role in the implementation of the mechanisms of biochemical adaptation, because it is an integral enzyme of erythrocyte membranes involved in the regulation of cell ionic homeostasis. Given the fact that ATPase is highly sensitive to composition of the lipids in the microenvironment of the enzyme and to their physical and chemical state, it is important to study the effect of ozone on the activity of ATPase of erythrocytes under the blood loss.

The **purpose of this study** was to investigate the dependence of the activity of ATPase of red blood cells from blood loss, as well as the possibility of correction of the detected changes by ozone administration.

Material and methods

Blood loss was modeled by taking 3 ml of blood from the caudal artery in rats. The animals were divided into 2 groups (n=15 in each group). Within 1 hour after blood loss we carried out transfusion of erythrocyte mass obtained by erythrocytes mixing with saline (group 1 – control of transfusion) or with ozonized saline (group 2 – experimental group). The concentration of ozone in the saline solution was 2 mg/l. The saturation of the saline with ozone-oxygen gas flow was made immediately prior to mixing it with erythrocyte mass by ozone generator "UOTA-60-01-Medozon" (Russia). Blood sampling for analysis was carried out during the blood loss and after 1, 24 h and 5 days after modeling of this exposure.

Results

In group 1, the activity of ATPase decreased during 24 h after transfusion of erythrocyte mass (to 79% from initial level) with recovery of the parameter to the values of intact animals in 5th day after manipulation. Transfusion of ozonized erythrocyte mass to experimental animals (group 2) led to initially reducing of the activity of ATPase (at 11% to initial level) with the recovery to the level of intact animals after 24 h of the experiment and subsequent increase in enzyme activity on 5th day of the post-transfusion period (at 23%). Given that inflammation mediators, such as derivatives of lysophospholipids (arachidonic acid and its metabolites), inhibit the activity of ATPases, it can be assumed that these processes take place in blood loss. In addition, ATPase is not only an ion pump, but also a receptor that binds digitalis-like factors and transmits a signal into the cell. An increase in endogenous "digoxin-like factors" in blood loss, as a typical stress reaction, will also determine the decrease in enzyme activity. At the same time, a less pronounced decrease in the activity of ATPase and its subsequent growth under the action of ozone proves the direct

effect of ozone on the enzyme activity and may be due to the optimization of pro - and antioxidant systems of cells.

Conclusion

Thus, the effect of ozone on the activity of ATPase in blood loss can be considered as a compensatory adaptive reaction of red blood cells, aimed at preventing violations of ion homeostasis of cells. The increase in the activity of ATPase is probably associated with the optimization of intracellular processes and the activation of glycolysis.

Key words: ATPase, ozonised saline, blood loss

THE EFFECTS OF THE INHIBITORY PEPTIDE ZIP AND A DONOR OF NITRIC OXIDE ON THE PROCESS OF RECONSOLIDATION OF CONTEXTUAL CONDITIONING IN TERRESTRIAL SNAIL

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To present, the idea has been formed that long-term processes of memory formation are associated with a new protein synthesis. It has been shown that it may happened the memory amnesia, if consolidated and stable long-term memory (LTM) will be reactivated prior to amnesic influence. It has been found that isoform of brain-specific protein kinase C, known as Mzeta protein kinase (PKMzeta), is necessary for learning. A significant part of these experiments was carried out using Zeta inhibitor peptide (ZIP), including learning on mollusks. Participation in the learning processes of nitric oxide (NO), which is currently considered as a new signaling molecule, is also shown. Thus, the research of the role of protein kinase PKMzeta and NO in the formation and reconsolidation of LTM is an actual task of neurobiology.

The terrestrial snail *Helix lucorum* was chosen as an object for experiments. It was formed the conditioned situational reflex (CSR), when the animals could distinguish the test signals applied in different situations (on a ball and flat surface). For the formation of CSR snails for 5 days showed 5 electrical stimuli per day when they are on the ball. Behavioral reactions were tested in 2 contexts: 1) on a ball (i.e., in standard conditions), 2) on a flat surface (i.e., in conditions other than standard). It was demonstrated a significant increase in defensive reactions when snails was on the ball, which demonstrates learning. The day after the test, a session of "reminders" was conducted and then immediately blocked protein biosynthesis by injection of anisomycin (AN). The results show that the reconsolidation of LTM occurs not only after a complete blockade of protein biosynthesis by AN after a reminder of the conditioned stimulus, but also after blockade of the protein kinase PKMzeta and similar protein kinases, by inhibitory peptide ZIP. The obtained results showed that the NO donor sodium nitroprusside don't affect the disruption of memory reconsolidation process, caused by ZIP, but at the same time inhibit the disruption of the memory reconsolidation process, caused by anisomycin.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University (No 17.9783.2017/8.9) and Russian Foundation for Basic Research (grant 18-015-00274_a).

Key words: nitric oxide, learning, long-term memory, proteinkinasa PKMzeta, reconsolidation of memory

ASSESSMENT OF OXIDREDUCTASE ACIDITY IN BLOOD OF BURN RATS EXPOSED TO EXOGENOUS NO-THERAPY IN EXPERIMENT

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At the heart of exogenous NO-therapy lies both the effect of the cold-plasma flow and the properties of the nitric oxide molecule (NO) itself. This compound is known for its antibacterial properties, and it is also a universal regulator of metabolic processes. Usage of pharmaceuticals to regulate the synthesis of NO in the body is associated with the occurrence of a number of side effects; therefore, an additional search for noninvasive physical regulators of nitric oxide is needed, and an assessment of their effect in the treatment of burn wounds.

The **aim of the study** was to evaluate the specific activity of oxidoreductases in blood of subjected to combined thermal trauma rats, also the effect of daily 14-day course of exogenous NO-therapy on the burn wound.

Material and methods

The work was performed on 30 Wistar rats weighing 250 grams divided into 2 experimental and 1 control (intact rats) groups. In the experimental groups animals suffered from the combined thermal trauma (CTT): a contact thermal burn injured on an area of 20% body surface (3 sec.) in combination with thermo-inflammation trauma (10 sec.) under anesthesia. In the first group (n = 10) were rats with CTT. In group 2 (n = 10), after the burn, the «PLAZON» apparatus (Russia) was used for production of NO-containing cold plasma flow during 14 days. The NO concentration on the axis of the plasma flow was ~ 3000 ppm. Duration of the procedure is 2 minutes. The animals were withdrawn from the experiment on the 14th day after the burn application. In the blood of rats, the activity of aldehyde dehydrogenase (AIDH), lactate dehydrogenase in direct reaction (LDHd), and lactate dehydrogenase in reverse reaction were determined (LDHr). The results of the studies are processed using Student's t-test.

Results and discussion

As a result of the study, we were found decrease of the specific activity of LDHd in 2.44 times ($p = 0,023$), LDHr in 1.65 times ($p = 0,031$), at CTT compared respectively with the control. The decrease in LDH activity may contribute to the accumulation of lactate in tissues of the burned rats, which is an indirect indicator of tissue hypoxia. It was noted that under the influence of exogenous NO-therapy, activity of LDHd increased in 1.78 times ($p = 0,029$) compared to burned rats. In this case, the specific activity of LDHr under the action of exogenous NO increased in 1.87 times ($p = 0.032$) compared with CTT animals. It was shown that the specific activity of AIDH decreased in 1.85 times ($p = 0,033$) in comparison CTT with the control. Reducing of

the enzyme activity leads to accumulation of a large amount of aldehydes, which are toxic to burned rats organisms. However, due to the application of exogenous NO-therapy to the burn wound of rats, the specific activity of AIDH in the blood increased in 5,98 times ($p = 0,035$) compared to animals with CTT.

Conclusion

It was revealed that the application of exogenous NO-therapy during 14 days to rats with the burn wound of CTT caused to an increased specific activity of LDHd and LDHr, as well as AIDH compared with burned animals.

Key words: exogenous NO-therapy, oxidoreductase, burn wound

VOGH-KOYANAGI-HARADA DISEASE

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Definition

Uveitis is the most remarkable ocular sign of the Vogt-Koyanagi-Harada syndrome (VKH), an autoimmune uveo-meningeal disorder characterized by bilateral ocular inflammation, serous retinal detachment, extra ocular irritations e.g. meningismus, neurological signs and skin manifestations.

Background

The Persian ophthalmologist Ali Ibn Isa was the first who reported the VKH pathology in the 10th century, he described the whitening of the hair, eyebrows, and eyelashes and the physical appearance of ocular inflammation. In 1906 Vogt and Koyanagi, described a condition characterized by bilateral iridocyclitis, uveitis and meningoencephalitis associated with vitiligo, alopecia, poliosis and auditory manifestation. In 1926, Harada reported a case with primary posterior uveitis associated with retinal exudative detachment and the integumentary manifestations previously mentioned. Since 1951, the medical literature has agreed to name this disease as Vogt-Koyanagi-Harada Syndrome (VKH).

Epidemiology

The incidence and prevalence of VKH syndrome varies worldwide. It has been strongly linked to susceptible populations like Asians, Afro-Americans, Hispanic and dark-pigmented skin Caucasians. In Japan, it is 15 per million habitants prevalent and the incidence is 6,5 cases per million.

VKH syndrome affects mainly in the third and fourth decade of life, with a slightly higher incidence in women. It is extremely rare in childhood. Monozygotic twins' cases have been described simultaneously in the same city; it shows that it is probably influenced by genetic factors or viral etiology.

Etiopathogenesis

Cytomegalovirus and Epstein - Barr virus have reported allergic mechanisms and viral infections. And also anti-retina, anti-choroidal and anti-cochlear circulating autoantibodies, as well as HLA II (DR1, DR4, DR53, DQA1, DQB1)

Clinical Features

TheVKH syndrome is divided in 3 phases:

Phase I The Prodromal: precedes common ocular symptoms and usually manifests with headache, low-grade fever, intense ocular pain, photophobia and vitiligo. The prodromal phase of VKH syndrome must be considered likely to manifest variable duration.

Phase II Ophthalmologic and Neurologic: It manifests bilateral uveitis, it may start affecting one eye first. Symptoms comprise blurry vision, photophobia, dysacusis and meningismus. Corneal keratic precipitates, small nodules on the pupillary border and papilledema with non-hemorrhagic retinal detachment; all these signs are manifestations of a granulomatous reaction in the ocular uvea.

Phase II can be prolonged for weeks or months. The neurological involvement can occur before, during and after ocular involvement and have a variable location and severity. It may be manifested by a meningoencephalitis with headache, meningeal signs, quantitative and qualitative commitment of conscience; cranial nerves detachments mainly II and VIII, and may compromise III, IV, V, VI and VII cranial nerves.

The involvement of the VIII nerve causes neurosensory hearing loss, sometimes bilateral and variably associated with tinnitus. There are also some endocrine disorders caused by hypothalamic inflammation such as amenorrhea, pituitary dysfunction, diabetes insipidus, hyperglycemia and hypocholesterolemia.

Sometimes it is possible to confuse the symptomatology with intracranial hypertension since it can cause headache and vomit. Nevertheless, intracranial pressure is usually normal and only a small percentage is subtly elevated.

The dermatological findings are alopecia, vitiligo, and poliosis, all of which may appear before, during or after ocular involvement.

Phase III Convalescence: this phase usually lasts from weeks to several months or continues manifesting by recurrent episodes of uveitis, dysacusis, poliosis, vitiligo and alopecia. The ocular test can reveal depigmentation of the perilimbal area.

VKH is classified in three distinct categories:

- 1): There is ocular involvement with no compromise of ear or skin that makes VKH suspect.
- 2): Ocular findings. Auditory and integumentary manifestations.
- 3): Ocular signs with two or more other systems.

Clinical case

A 35-year-old Hispanic woman, without remarkable medical history, started her treatment in May 2015 with decreased bilateral visual acuity and greater involvement in the left eye. Conjunctival hyperemia, bilateral retroocular pain, otalgia, moderate to intense bitemporal headache, with progressive loss of visual acuity and remissions and exacerbations of symptoms.

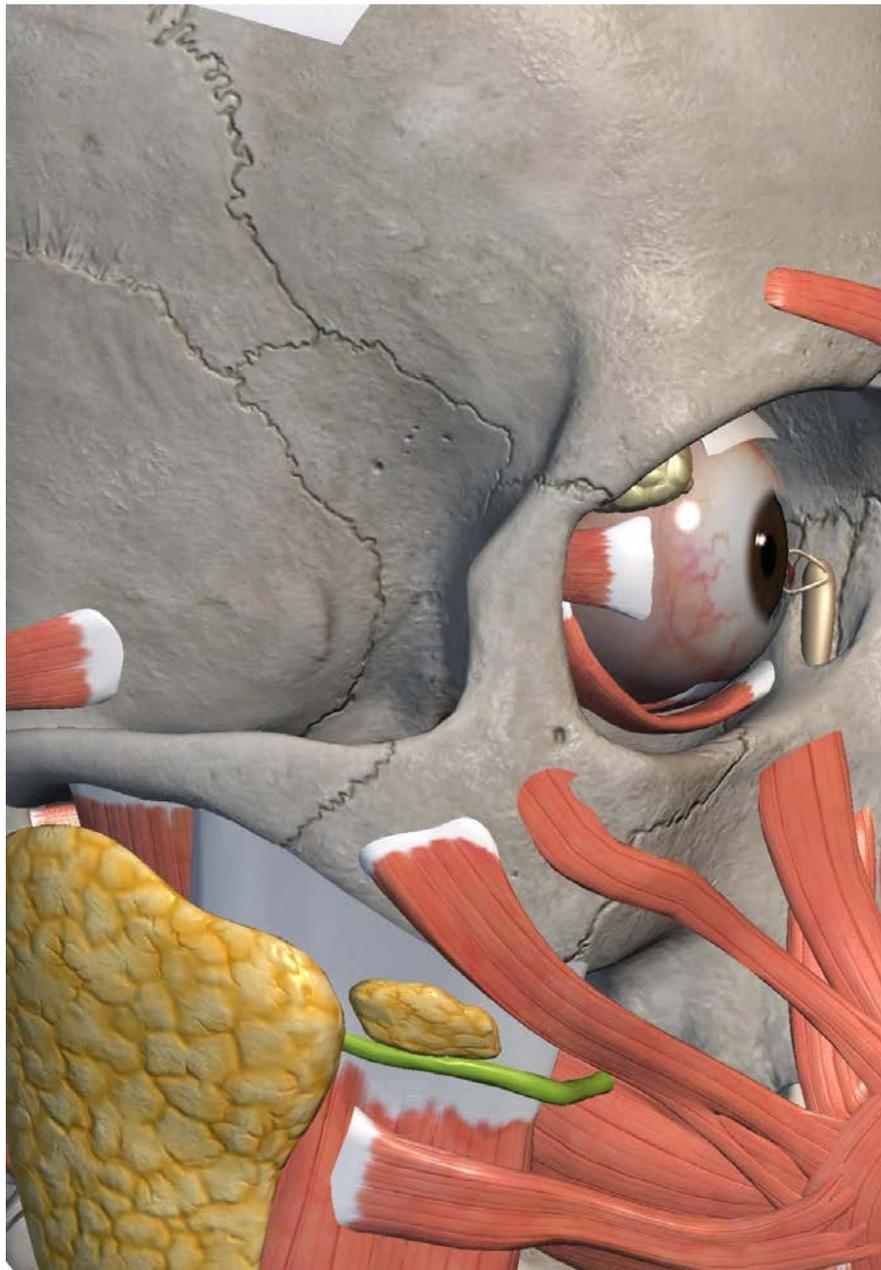
At the initial treatment, the patient was prescribed oral Prednisone 100 mg daily, bilateral betamethasone weekly, Azathioprine 50mg, improving in visual acuity and Optical Coherence Tomography (OCT) confirmed folding of the retina in both eyes. After a month of ongoing treatment, the patient suffered a relapse, presented serous retinal detachment in both eyes and exacerbations of meningeal symptoms.

It was decided to prescribe the patient with ozone therapy treatment, retroocular and major autohemotherapy; Maht 20 micrograms per 100 ml (Application every 5 days) and local 5 micrograms per milliliter, 2ml. The patient showed symptomatic improvement the next day. Coherence Tomography (OCT) confirmed adhesion of the retina in both eyes.

The initial treatment and ozone therapy weekly continued in the prescription. Betamethasone ocular treatment was withdrawn and steroid reduction was initiated oral via.

Chart 1.1 Exams done before treatment

Type	Exams	Results
Immunology	Ultrasensitive C-Reactive Protein.	• 1.42 mg/L V.R. 0-5
	Native double-stranded Anti- DNA Antibodies.	• Negative 24.86 UI/ml
	Anti-Cardiolipin Antibodies, IgG and IgM	• Anti antibody Cardiolipin IgG (Negative 1.54GLP/MI) • Anti antibody Cardiolipin IgM (Negative 1.7 MLP/MI)
	Antibodies Anti-Smith (Sm)	Negativeless than 2.0 U/mL
	Antibodies vs I and II Herpes Simplex IgG and IgM	• Antibodies vs Herpes Simple 1 IgG (0.73 UI/ml) • Antibodies vs Herpes Simple 1 IgM (0.54 UI/ml) • Antibodies vs Herpes Simple 2 IgG (0.47 UI/ml) • Antibodies vs Herpes Simple2 IgM (1.25 UI/ml)
Antibodies vs Toxoplasma IgG and IgM	• Antibodies vs Toxoplasma IgG (0.02) • Antibodies vs Toxoplasma IgM (0.11) • Antibodies Antinuclears (Negative)	



Fluorescein Angiography. Optical Coherence Tomography (OCT)

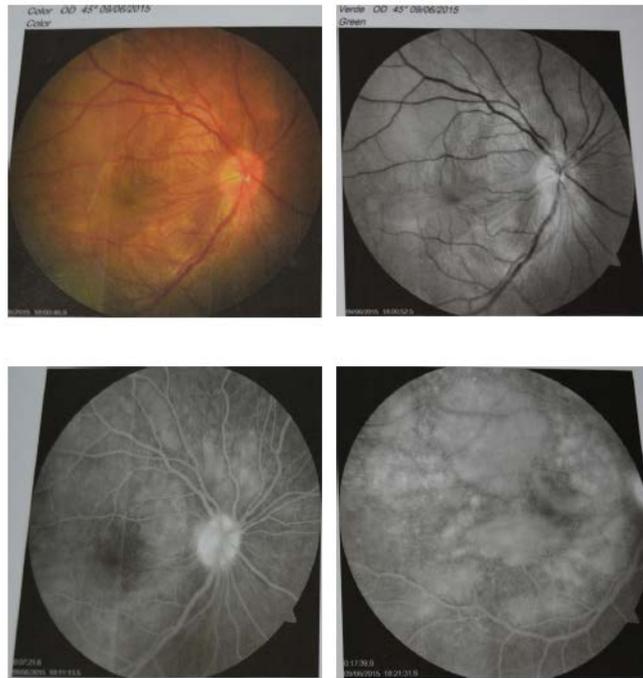


Fig.1.1 Early and progressively increasing hyperfluorescence, spreading and blocking the sub retinal area.

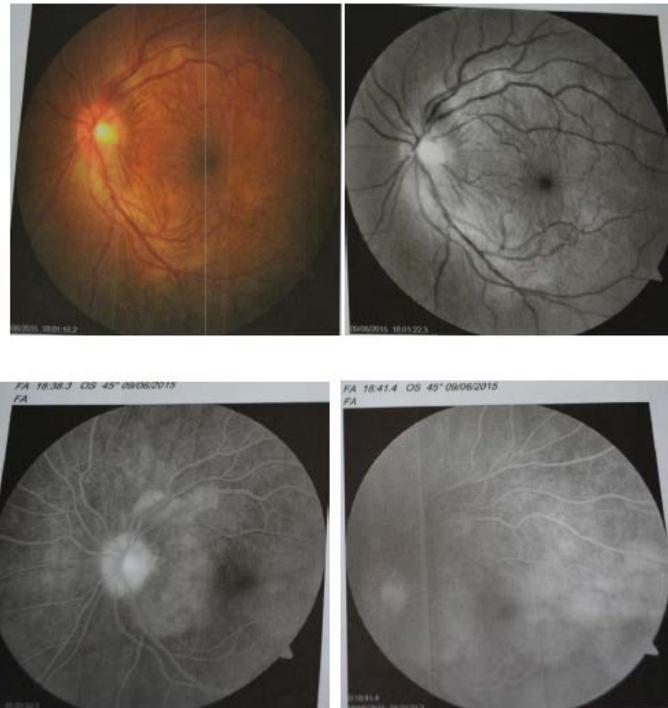


Fig. 1.2 White-yellow lesions are observed in the left eye.

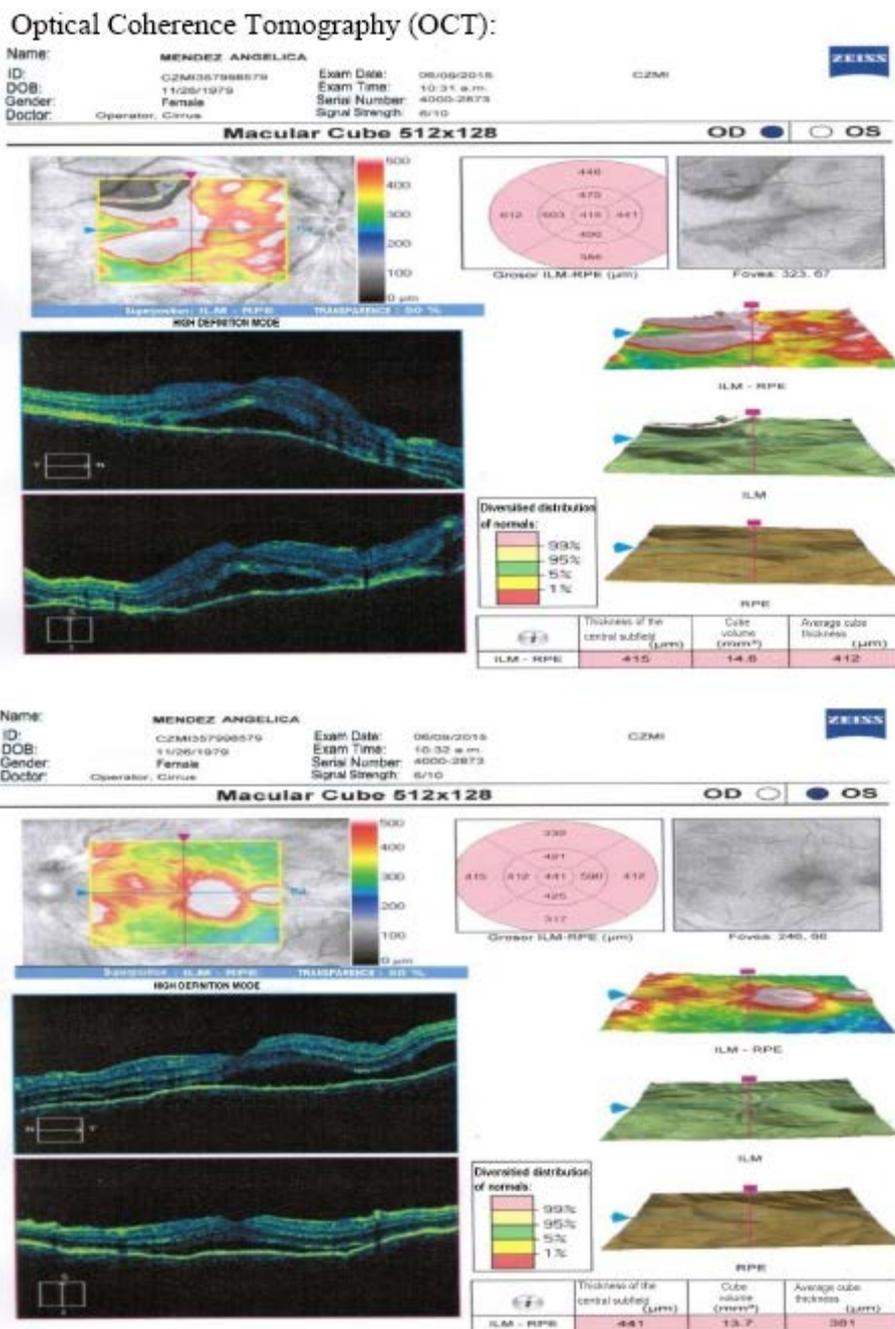


Fig. 1.3 Serous detachment of the retina.

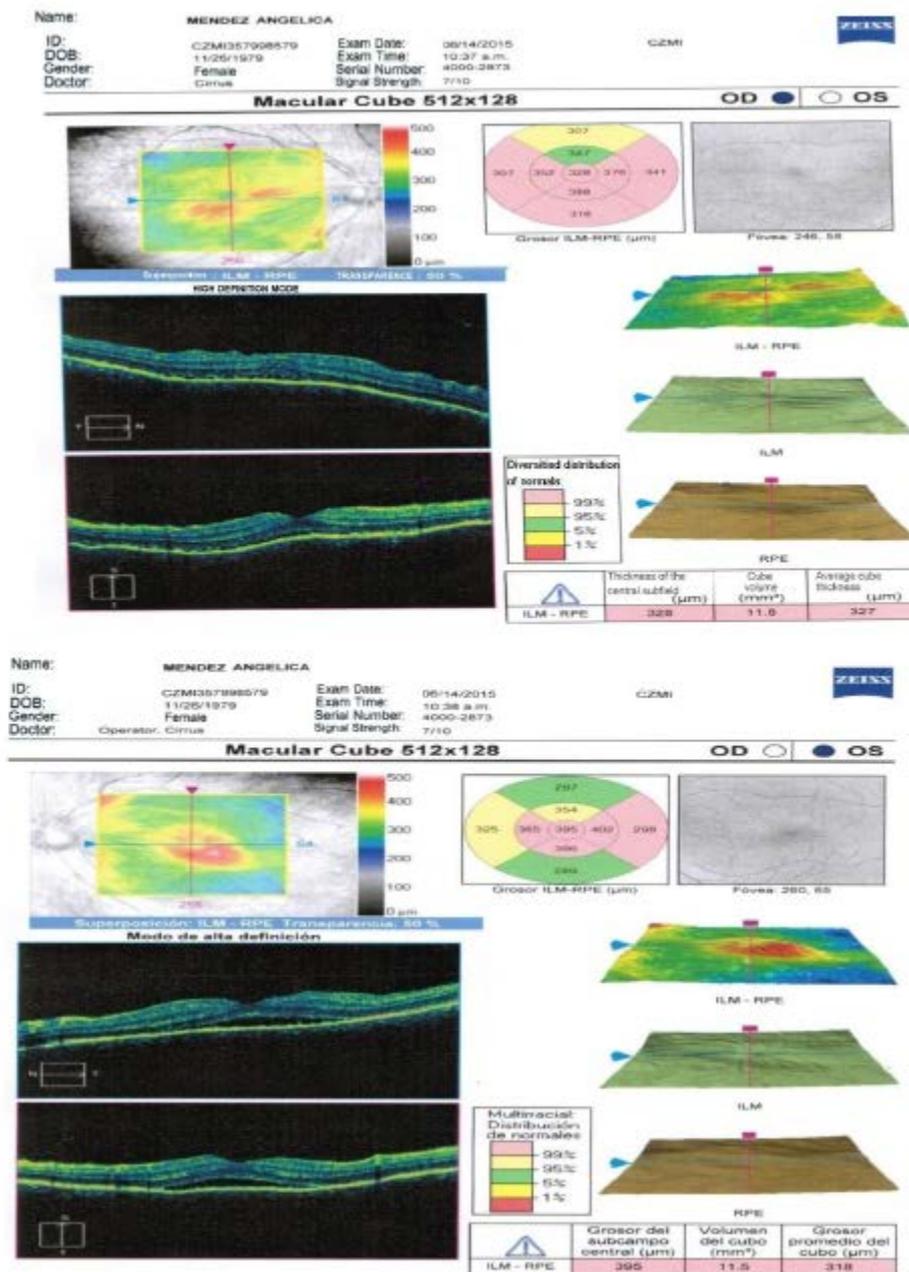


Fig 1.4 The adhesion of the retina is more observed in the right eye. After treatment with oral Prednisone and Azathioprine.

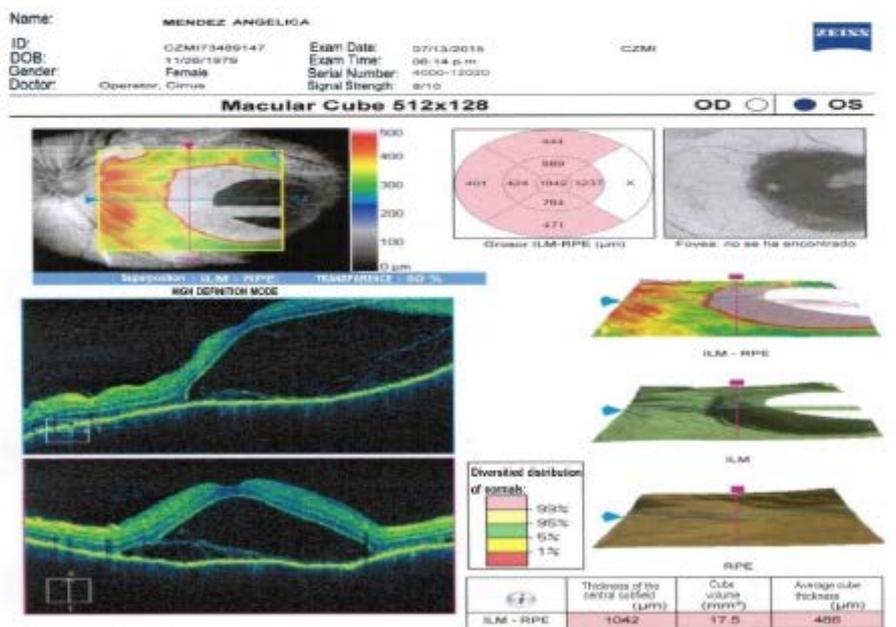
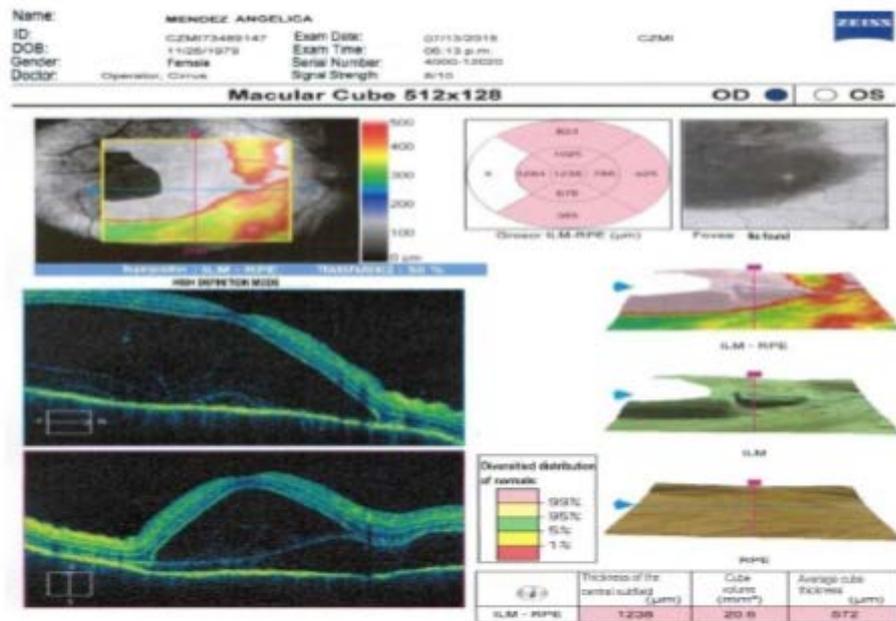


Fig 1.5 Serous detachment of the subfoveal retina.

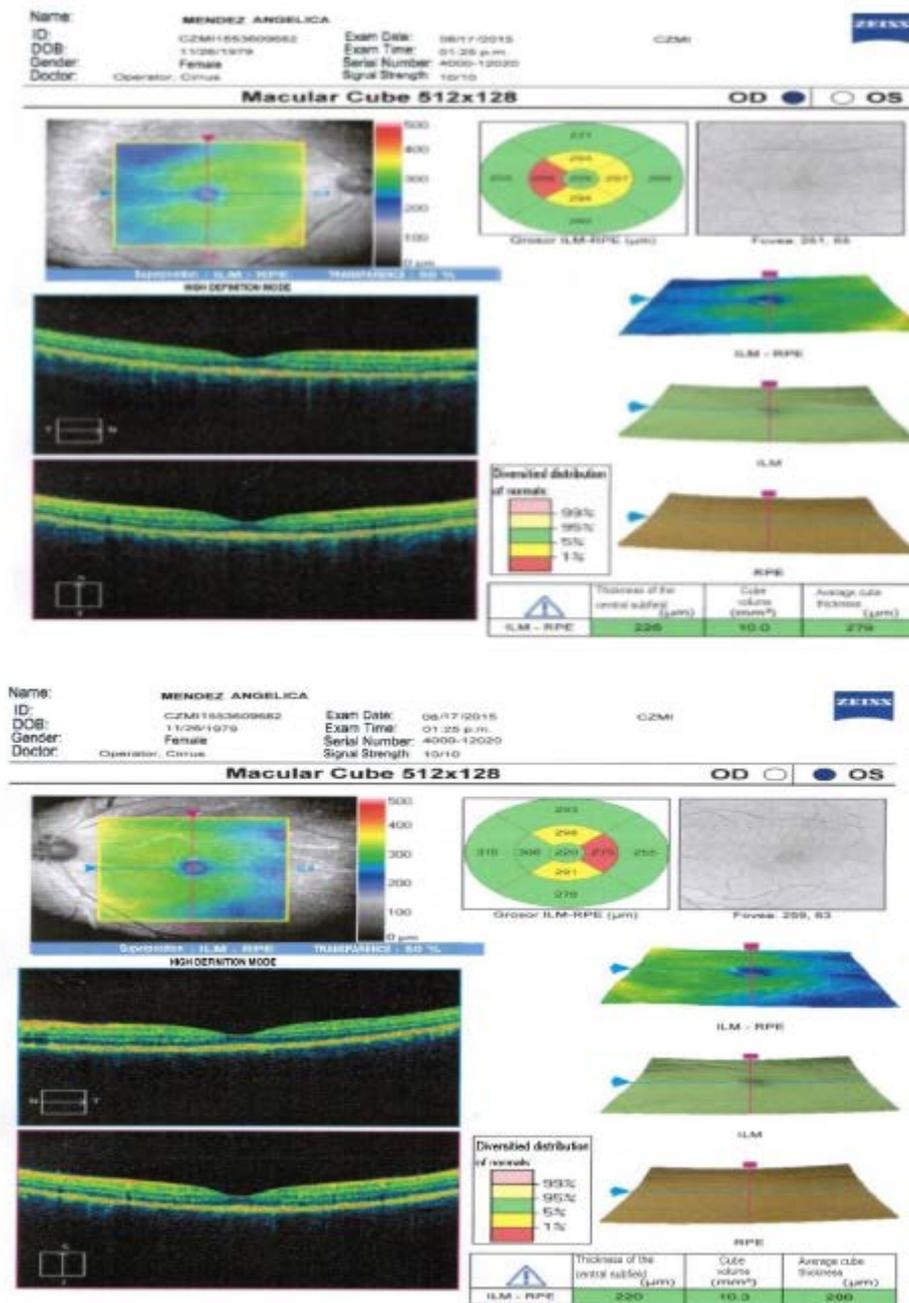


Fig 1.6 Total retinal folding after treatment with ozone therapy.

Discussion

We described a patient with an uncommon case of VKH, since she does not present signs in the anterior segment of the eye, only bilateral retinal detachment and meningeal symptoms.

The spectacular response, almost immediate to ozone therapy, corroborates its anti-inflammatory and immuno modulatory action.

The cause of VKH disease is still unknown, there are no specific tests to diagnose this disease.

VKH syndrome diagnosis is supported by a suggestive combination of the clinical signs of bilateral retinal detachment and uveo-meningeal signs.

In literature, therapeutic schemes are proposed such as: oral steroids, intravenous steroids and regional cortico steroids, cyclosporine, antimetabolites and alkylating agents.

In recurrent cases (which are not few, just not published) cyclosporine, chlorambucil, cyclophosphamide, azathioprine, methotrexate, tacrolimus, mycophenolate, adalimumab and rituximab even endovenous immuno globulins have been used with varying effectiveness.

In general, we do not know an effective treatment without or with minor risks for this disease. The answer can be the ozone therapy, we know its anti-inflammatory, immune modulatory effects and its harmlessness.

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Key words: Vogt-Koyanagi-Harada syndrome, ozone therapy

THE ALDEHYDE DEHYDROGENASE ACTIVITY IN HEART OF RATS WITH COMBINED THERMAL INJURY UNDER THE INFLUENCE OF DINITROSYL IRON COMPLEXES

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Introduction

It is known that severe thermal trauma is complicated by the early development of the syndrome of multiple organ failure and endogenous intoxication. In particular, the content of highly toxic aldehydes is increased. The aldehyde dehydrogenase (AIDH) plays the main role in the utilization of which. Recently nitric oxide is widely used in the treatment of a number of diseases, but the role of dinitrosyl iron complexes (DNIC) in the metabolism of aldehydes and their effect on oxidoreductase activity in burns has been poorly studied.

The aim of the work was to study the activity of AIDH in the heart of rats with combined thermal injury under the influence of DNIC.

Material and methods

The experiment was performed on Wistar rats. Three groups of rats were formed: the first - control (healthy animals, n = 15); the second - animals with a burn (n = 15), which every day intraperitoneally were injected by 1 ml of saline solution; the third - animals with a burn daily treated an intraperitoneal injections of a 10% solution of DNIC (1 ml, 0.3 mmol / l) (n = 15). DNIC with a glutathione was prepared by Vanin's method. The concentration of DNIC was determined spectrophotometrically on a Power Wave XS spectrophotometer (Bio-Tek, USA) in the wavelength range 410-700 nm. The combined thermal trauma was applied under anesthesia (Zoletil + Xylon). The activity of AIDH was evaluated in heart homogenate. The results of the studies were processed using Student's t-test.

Results and discussion

It was shown that the specific activity of aldehyde dehydrogenase in the heart significantly decreased by days 3 after the burn by 38% in comparison with healthy animals. There was a decrease in AIDH activity by 28% compared to healthy animals by days 10 after the burn. Thus during the thermal trauma the activity of aldehyde dehydrogenase decreased on the 3rd and 10th days, the greatest fall was observed on the 3rd day after the burn.

The introduction of dinitrosyl iron complexes in the thermal injury had a statistically significant effect on the activity ALDH in heart. Trends were noted to increase the activity of the enzyme at day 3 to 10% after injury (compared to burn) and a statistically significant increase of 23% on day 10 compared to untreated animals with thermal trauma.

Conclusion

Thus a decrease in the activity of aldehyde dehydrogenase in the heart during thermal trauma was shown. The normalizing effect of DNIC on the catalytic properties of aldehyde dehydrogenase was revealed.

Key words: burn, aldehyde dehydrogenase, nitric oxide

MEDICAL OZONE IN THE REHABILITATION OF WOMEN OF REPRODUCTIVE AGE AFTER RADIOWAVE SURGERY FOR NON-MALIGNANT DISEASES OF THE CERVIX

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The **aim of the investigation** is to increase the efficiency of treatment of non-malignant cervical lesions in women of reproductive age using modern technologies (radiosurgery and ozone therapy).

The study group consisted of 82 women aged 17 to 48 years. Clinical, laboratory and special methods of examination were used, on the basis of which the diagnosis was made of chronic cervicitis (endo-and EC - zocervicitis with ectopia)-44 patients, CIN II - 25 women, para-and hyperkeratosis, acanthosis of multilayered planar epithelium (including leukoplakia without atypia) - in 13 patients. Used radiosurgical device "Surgitron" company "Ellman international" (USA), which is a high frequency electrosurgical instrument with the output frequency of 3.8 MHz, which enables non-contact dissection of tissue. All radiosurgical procedures were performed after the above examination on the 5th-9th day of the menstrual cycle. In the absence of contraindications every other patient went on a course of rehabilitation therapy by medical ozone. 41 women (group 1 study) in two weeks of the radiowave surgical procedure and the separation of the eschar, to pursue a course of ozone therapy included the infusion of ozonized physiological solution with ozone concentration of 1.5 mg/l, in combination with vaginal irrigations of ozonized distilled water with the concentration of ozone 3 mg/l, with a gradual decrease in ozone concentrations up to 1.5 mg/l. For obtaining ozonized solutions were used the apparatus "Medozon" (Russia). The course consisted of 5-7 daily sessions, depending on the degree of epithelialization. The second group of patients - 41 women after radiowave excision did not receive any additional therapy. Control examination of patients after treatment was carried out in 1, 3, 6, 9, 12 months. The status of the cervix was evaluated using clinical, cytological and colposcopic study. After the radiowave treatment stenosis, strictures, disorders of cervical architectonics, bleeding, with the exception of minor blood secretions in the rejection of the scab, was not observed in any case. Full epithelialization one month after treatment in the first group of women was revealed in 43% of women cases, in 3 months – in 50%, in 6 months – 3.5%, in 12 months – 3.5% of women. In the second group of women, cervical epithelialization was significantly slower: one month after treatment, complete epithelialization was noted in only 13% of women, 3 three months – in 29%, 6 months – in 9.7% of women, 9 - in 13%, 12 months– in 3.2% of women. The conducted research testifies to expediency and high efficiency of ozone therapy in patients with non-malignant diseases of the cervix uteri after radiowave exposure. A simple method of ozone therapy used after radiosurgical treatment, increases the

treatment efficiency through the elimination of concomitant nonspecific inflammation and activation of epithelization processes due to bactericidal, virusolitical, reparative effects. Ozone therapy like intravenous infusions and vaginal irrigation with ozonated solutions after radiosurgery allows to optimize treatment of non-malignant diseases of the cervix uteri in women of reproductive age.

Key words: non-malignant cervical lesions, radio-wave exposure, ozone therapy

EPR STUDY OF NITRIC OXIDE PRODUCTION CHANGES IN TISSUES OF HEART AND LIVER OF RATS WITH THE 30-DAY RESTRICTION OF MOTOR ACTIVITY AND SUBSEQUENT RECOVERY

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Hypokinesia (restriction of motor activity - MA) is one of the most important medical and social problems caused by lifestyle, professional activity, a long bed rest, etc. Thus there is a decrease in loading on the muscular apparatus, consumption of oxygen by tissues and activity of oxidative processes significantly decrease, there are changes of contractile function of a heart muscle. Nitric oxide (NO) plays an important role in the adaptation to various conditions in external environment. Based on this, the aim of the study was to study the possible changes in the production of NO in the tissues of the heart and liver of rats, which were restored within 2 weeks after a long (30 days) restriction of MA.

White outbred rats of different age were used for experiments. The restriction of MA of growing rats was achieved by placing them in pen-case cages. Limitation of MA started from age of 20-day-old rats and to 25 day limitation of MA stay animals in pen-case cages made up 23 hours. We studied the changes in the amount of NO in the tissues of the heart and liver using the electron paramagnetic resonance (EPR) spectroscopy method. It was applied the technique of spin traps, which allows to detect NO in small concentrations used the complex of Fe²⁺ with diethyldithiocarbamate c (DETC). EPR measurements were carried out in the X-band on the EPR spectrometer ER-200 by Bruker at a temperature of 77 K. The number of NO was estimated by the intensity of the characteristic EPR signal belonging to the complex (DETC)₂-Fe²⁺-NO. We found that the amount of NO formed in the tissues of the ventricles and atria of the heart increases after a 30 - day restriction of MA by 2-3 times. It was found that 2 weeks after recovery of MA, the level of NO production in the heart tissues decreases even more.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University (No 17.9783.2017/8.9).

Key words: nitric oxide, heart, hypokinesia, electron paramagnetic resonance

HEMODYNAMIC EFFECTS OF OZONE-BACTERIOPHAGOTHERAPY OF CHRONIC ENDOMETRITIS

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Introduction

One of the urgent problems of modern gynecology is chronic endometritis (CHE), which is a frequent cause of infertility, miscarriage, and failures in the implementation of assisted reproductive technologies. Ophthalmic bacteriophage therapy, combining antimicrobial effects of phages and ozone with its known hemorheological effects, is a promising method of complex effect on the homeostasis of patients with CHE.

The aim of the study was to establish the nature of the effect of ozone-bacteriophage therapy on the blood flow in the uterine artery basin in patients with chronic endometritis.

Material and methods

100 patients aged 24-42 years with a diagnosis of chronic endometritis were examined. Group I (50 patients) received 5-7 intrauterine irrigations with ozonated saline followed by administration of the preparation of piobacteriophage into the uterine cavity. Group II (50 patients) received traditional treatment in the form of broad-spectrum antibiotics, vitamin complexes, microelements and physiotherapy. All patients underwent ultrasound of the pelvic organs and dopplerometric study of hemodynamics of uterine vessels on the 19-24 day of the cycle before treatment and 1 month after its completion.

Results

Source the majority of patients by ultrasound scanning in the period of "window of implantation" was marked hypoplasia of the endometrium, hypovascularization one of the walls of the uterus, disorders of blood flow at the level of arcuate, radial, basal arteries, spiral arteries were visualized in less than 1/3 of patients.

As a result of ozone-bacteriophage therapy, in 80% of patients it was found that there was an increase in the frequency of visualization of spiral arteries and the restoration of blood flow in vessels of all calibers (from uterine arteries to spiral arteries), and an increase in the thickness of the endometrium by 48,2%. In group II, the majority (78%) of women continued to have insufficient blood supply and signs of endometrial hypoplasia.

Key words: chronic endometritis, ozone-bacteriophage therapy, blood flow

COMPLEX LABORATORY MONITORING OF LOCAL OZONE THERAPY USING IN DERMATOLOGICAL PRACTICE

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Introduction

Successful clinical using of local ozonids-containing preparations in different fields of medicine (dermatovenereology, gynecology, stomatology, combustiology) last 2-3 decades needs pathogenetic rationale. Wide production of local drugs contained of chemical modified ozonized olive oil began only several years ago in several countries (Russia, Ukraine, Italy, Cuba, Spain, Turkey, USA, Mexico). One of perspective variant of its using is trichology. Relevance of such preparations on trichological market is positioned with powerful antioxidative effect and restoration of functional activity of cells and de novo formation of hair follicles. Many modern local drugs (peptide preparations, Selencin, Quilibe line) introduce on activation of oxygen provision of hair follicle. Improvements of microcirculation during local ozone therapy of different skin diseases including scalp involvements have been demonstrated several times with laser Doppler flowmetry. Such estimation of treatment efficacy of different dermatoses was conducted in psoriasis, lichen ruber planus, atopic dermatitis, lichen sclerosus et atrophicus, diffuse alopecia, alopecia areata.

Aim of investigation: carrying out of experiments in vitro и in vivo as demonstration of influence of new ozonids-contained local preparations on hair and scalp skin condition in dynamic.

Materials and methods

For investigation of ozonids-contained cream-mask 1500 mcg from Ozodermis series influence in vitro condition we used trichoscope Aramo SG with lens for enlargement of hair imagination in 60, 200 and 1000 sizes. Initial photos, photos in dynamics after expositions of local preparations during 15, 30 and 45 minutes have been used for analysis.

For study of scalp area condition in course treatment with cream-mask Ozodermis 1500 mcg in 12 volunteers (6 males and 6 females) we measured control points in parietal and occipital lobes (tattoo tags fixation) before application, after 15 and 30 minutes of application, and after 6 weeks of applications 3 times a week during 15 minutes. Control complex estimation used trichoscopy, phototrichogramme, lase analyzer of microcirculation with laser Doppler flowmetry method and diagnostic combine for study of functional parameters of skin Multi Skin Test Center MC 1000 (corneometry, sebummetry and elastometry sensors). Volunteers were 22-23 years without any diseases of scalp skin. Every investigation with laser Doppler

flowmetry was during 400 seconds with probe mm in diameter in red channel of laser radiation (wavelength 0,63 mcm, probing depth about 0,8 mm), and also in infrared channel (wavelength 1,15 мкм, probing depth about 1,6 mm including deeply located microvessels). The analysis of the received LDF data allowed us to evaluate the area's state of skin microcirculation in perfusion units, as well as to estimate the microcirculation's control elements quantity: the active control factors (endothelial, myogenic and neurogenic mechanisms of vessels wideness regulation) and the passive control factors (respiratory and cardiac mechanisms). Statistical processing of the data was carried out by Microsoft XL.

Results

Trichoscopy in vitro in prolonged exposition before 45 minutes do not demonstrated damage effect of ozonids-contained preparation with modified olive oil. Dynamic complex instrumental study in 6-weeks using of cream-mask Ozodermis with peroxide value 1500 mcg were more informative as phototrichogramme results with increment of hair density on sm^2 , enlargement of hair stem diameter, rising level of anagen hair. Microcirculation data on laser Doppler flowmetry method had tendency to elevation. In 50% of group after 6 weeks of application we note decrement of sebummetry parameters.

Conclusion

Local application of ozonids-contained preparations in trichology has perspectives in alopecias connected with disorders of oxygen metabolism (alopecia areata, diffuse reactive alopecias) and in seborrheic dermatitis at the expense of suppression of yeast-like fungi as a result of fungicidal activity of ozonized olive oil. Laser Doppler flowmetry using for estimation treatment efficacy has value in local therapy with ozonids-contained preparations as method of activation of tissue oxygen metabolism.

Key words: dermatology, local ozone therapy, laser Doppler flowmetry

FACIAL BIOSTIMULATION WITH PRP ACTIVATED WITH OZONE RESOUND ON CELLULAR REDOX BALANCE AND IMPROVES LIPOATROPHY IN HIV PATIENTS

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Introduction

Infection by both human immunodeficiency virus (HIV) generates sustained inflammation with increased reactive oxygen species production. Pathogenic impact of high-grade local and systemic oxidative stress indexes in antiretroviral treated patients are higher and recognized to influencing in lipodystrophy which is proposed can be ameliorate with platelet rich plasma (PRP). The aim of this quasi-experimental study was to determine the effect and safety of facial bioestimulation of PRP activated with ozone and calcium in lipoatrophy -aids Cuban individuals.

Methods

Blood samples were drawn from 30 individuals. Total peroxide (HPO), malondialdehyde and advanced oxidation protein products (AOPP) as damage indexes and antioxidant responses (glutathione, peroxidation potential, superoxide dismutase and catalase) were assessed. Also haematological and hemochemical indexes and progression markers (viral load, T CD4+ lymphocyte absolute count) were determined and also quality of life questionnaires. Different statistical analyses were done comparing baseline respect final values of variables after 5 interventions during a year.

Results

Reductions of lipoatrophy lesion at 75% of patients and significant differences in global indexes of damage and antioxidant status of 80% of patients at the end of the study was demonstrated ($p < 0,05$). The comparison revealed a significantly smaller damage (HPO, AOPP) and lower antioxidant status (SOD, CAT) compared to baseline values ($p < 0,05$). Non-significant modifications was observed in hematological and hemochemical indexes ($p > 0,05$) with 75% improves of two dimension in quality of life. Non adverse reactions were observed during study period.

Conclusions

These results corroborate that beneficial reductions of oxidative stress occurs in lipoatrophy aids patients during safety PRP-ozone facial bioestimulation with high perception of health improve by patients. Redox indexes diagnosis should be considering in early diagnostics, prevention and treatment with PRP, which would be worthwhile to conduct a more comprehensive study and manage of lipoatrophy.

Key words: facial bioestimulation, ozone, PRP

PRODUCTS OF OZONOLYSIS OF OLIVE OIL AND CREAM BASES AS A KEY FOR ITS SANOGENIC ACTION

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The aim of this work was the study of modification of the compounds from olive oil and cream basis during its ozonolysis, which causes its sanogenic activity.

Material and methods

We studied the composition of hydrophilic cream with reactive oxygen species is generated through ozonolysis by IR-spectrometry method, modifying for organic substances. In special spectrum field (4000 - 400 cm^{-1}) we tested a number of specimens (oleic acid, medical olive oil before and after its ozonation and cream basis is used for generation of ozonized products with different concentration of reactive oxygen species (3, 5, 10 and 30%). In all specimens were estimated pro- and antioxidant activity in cream basis and final products by Fe-induced bioluminescence method ("BHL-06" device, Russia). We also studied oxidative and restorative potential and pH of all specimens with standardized device "InoLab pH7110" (Germany). The level of reactive oxygen species was fixed by iodometric titration (ISO 3960-2013). Crystallogenic properties of indicated compounds were tested with teziocrystalloscopy technique (Martusevich A.K., Grishina A.A., 2009). Description of crystalloscopic facias was executed with special semiquantitative criterias. Additionally we registered dehydrogenase activity in studied compositions with laser diagnostic complex "LAKK-M" (Russia). Statistic calculations were provided by standard algorithms with Statistica 6.1 for Windows.

Results

Our studies allow to demonstrate the critical role of some organic substances in determination of sanogenic potential of creams with different concentration of reactive oxygen species. These molecules are ozonides and hydroperoxides is generating directly during ozonation. In addition, different aldehydes, azelainic, pelargonic and other short-chain organic acids also play an important role in positive bioregulatory effect of tested compositions.

Conclusion

In whole, sanogenic activity of creams is saturating by reactive oxygen species is associated with autocatalytic oxidative processes. They are induced by ozonation of cream basis.

Key words: ozone, olive oil, ozonolysis, metabolic potential, cream, reactive oxygen species

THE INTENSITY OF FREE RADICAL PROCESSES IN MITOCHONDRIA OF LIVER OF RATS DURING DEEP HYPOTHERMIA

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Deep hypothermia has been used in medicine for a long time with cerebral ischemia, stroke, cardiac arrest, hypoxia. However, in the case of an exsidental (unintended) hypothermia, the processes generating ATP are suppressed, which can lead to activation of free radical processes and oxidative stress. The purpose of our study is to study the intensity of free radical processes with deep hypothermia (body temperature 19-20 ° C) in mitochondria of rat liver.

The study was carried out on males of white, uncommon rats weighing 180-220 g. Hypothermia was caused in a cold chamber in which circulated water with a temperature of 4-5 ° C. The intensity of free-radical processes was determined from the content of oxidative modification products of lipids and malonic dialdehyde (MDA) proteins and carbonyl groups, also in the content of sulfhydryl groups (SH groups) of the protein fraction and reduced mitochondrial glutathione (GSH).

In the study of lipid peroxidation products and oxidative modification of proteins with deep hypothermia in rat liver mitochondria found elevated levels of MDA by 20% in the experimental group relative to the control group twice carbonyl groups relative to control. However, the level of sulfhydryl groups in the protein fraction of mitochondria did not change. Reduction of reduced glutathione in hypothermic rats is insignificant. Thus, the data obtained by us testify to the intensification of free-radical processes, while at the same time, the stability of SH-groups of proteins in mitochondria of rat liver is observed.

Key words: deep hypothermia, malonic dialdehyde, glutathione, free radical processes

THE INTENSITY OF FREE RADICAL PROCESSES IN SYNAPTOSOMES OF RAT BRAIN WITH ISCHEMIA AND REPERFUSION

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Introduction

Oxidative stress plays an important role in the ischemic damage of neurons, leading to their death. At the same time, cellular targets and the mechanisms that underlie radical-mediated damage to neurons at the level of synaptic contacts have not been adequately studied. In this paper, the effect of cerebral ischemia and subsequent reperfusion on the intensity of oxidative modification of lipids and membrane proteins, as well as the activity of antioxidant protection in the synaptosomes was studied.

Materials and methods of research

Male Wistar rats weighing 200-220 grams were used in the study. Brain ischemia was caused by the occlusion of two carotid arteries within 1 h, and reperfusion (1 h) by the removal of ligatures. All surgical procedures were performed under anesthesia (intraperitoneal administration of sodium thiopental in a dose of 40-50 mg / kg). False-operated animals served as controls. Synaptosomes from the cerebral cortex were isolated by differential centrifugation in a sucrose density gradient, and their membranes after osmotic shock. In the suspension of synaptosomes, the content of malonic dialdehyde (MDA), a marker of lipid peroxidation (LPO), was studied, and in synaptic membranes, the content of carbonyl groups, a marker of oxidative modification of proteins. Both their baseline and in vitro accumulation in Fenton were evaluated. In synaptoplasm, the content of reduced glutathione (GSH), activity of superoxide dismutase (SOD) and catalase was determined.

Results and discussion

1 hour after the restoration of the blood flow in the brain, the level of MDA in the synaptosomes did not differ significantly from the control level, and in the in vitro incubated in the Fenton medium, the amount of MDA in the suspension of synaptosomes increased by 78%. In proteins of synaptic membranes during reperfusion, the content of carbonyl groups increases by 49%, but the amount of carbonyl groups incubated with oxidants does not increase with respect to the control. Ischemia and subsequent reperfusion significantly suppress antioxidant protection of synaptic endings of neurons: GSH content is reduced by 63%, SOD activity by 64%, and catalase activity by 69% relative to control.

Conclusion

The data obtained show that ischemia / reperfusion stimulates the formation of reactive oxygen species in the synaptic endings of neurons, which leads to the oxidation of membrane lipids and proteins. At the same time, the oxidation of membrane lipids is significantly increased. The degree of oxidative modification of membrane proteins is also increasing. The development of oxidative stress is also evidenced by a sharp decrease in the content of GSH in synaptosomes. A significant decrease in the activity of antioxidant enzymes in ischemia / reperfusion can also be the result of their oxidative modification. In general, the obtained data indicate that in cerebral ischemia / reperfusion in the region of synaptic endings of neurons oxidative stress develops, which may be the reason for the violation of the synaptic function.

Key words: ischemia, reperfusion, synaptosomes, reactive oxygen species, oxidative stress

OZONE-SURGICAL DEBRIDEMENT IN TRAUMA SURGERY

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The material includes the best practices and other authors' data on staging surgical approach of battle wounds and injuries. In the ischemic tissues with propensity for the infectious complications the traumatic fascial compartment is formed, the syndrome which should be resolved immediately with the help of surgical and medicamental approach. It is not always possible to achieve the complete bacterial decontamination of wound process only with the help of surgical processing (debridement). Additional physico-chemical effects on the infected wound: negative pressure wound therapy (NPWT), ultrasonic cavitation, vulnerosorption includes the ozone as well, which was first used during the World War I to treat purulent wounds, fistulas, and burns.

In the trauma surgery of present day, the ozone therapy is presented as an option of adjuvant treatment- evacuative support. The assistance system with the developed method of ozone therapy is oriented to its staging implementation as an ozone-surgical debridement. The combination of local infiltration and regional ozone therapy with an ozone concentration of 1.0-3.0 mg/l blocks the tissue hypoxia, ischemia, sludge, and hypertensive compartment syndrome.

The range of emergency aid defines the stage of treatment- evacuative support. The paramount importance has the urgent delivery of the wounded and injured to the stages of medical care having the urgent ozone therapy in the form of blockades which simultaneously complements the surgical aid.

At the forward aid posts, in the conditions of the dressing room, during the primary surgical aid the anti-inflammatory ozone blockades should be implemented by the puncturing of tissues with the help of portable ozonizers.

In the reception and sorting module of field hospitals at the stage of qualified care during the primary surgical treatment, the sectional saturation of tissues with ozone accompanies the main elements of the debridement: discission, extirpation, fasciotomy, drain on a step-by-step basis.

In the in-patient hospitals of specialized base hospitals and traumatic centers, the ozone therapy can be used in complex treatment as a sorption method of detoxification. By the bandaging and surgical treatments, the wound cavities are drained with the active ozonation, by closing of tissue with ozonized oil. The treatment is supported by the chambered aerations with the ozone-aseptic environment.

Thus, at all stages, along with the surgical aid, the ozonation systematic method is successively implemented. By influencing the general and local stages of the wound process the conditions for

morphological zones regression are formed. Forming the effect of hydro-preparation, vacuum, antiseptic, adsorbent and drainage, the ozone-surgical debridement acquires the status of military-field ozone therapy in providing of urgent assistance during the evacuation stages.

Key words: stages of surgical approach, ozone-surgical debridement

COMBINATION OF REGIONAL ANTI-INFLAMMATORY AND OZONE BLOCKADES IN TREATMENT OF HAND AND FINGER INFECTION

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Treatment approaches of purulent diseases have historically changed over the years. However, surgical treatment remains the most important element of therapy. The radicalism of surgery is caused by the emergence of secondary necrosis. From the pathogenetic point of view, the tissue hypoxia and the histolytic effects of microbic enzymes determine formation of necrotic tissues. At purulent septic diseases and re-infection, we developed the methods of Novocain blockades using various antibiotics and antihypoxants for the purpose of secondary necrosis prevention. In the instructions for military field surgery, there is one of the formulas for such blockades: 10 ml of 0.5% novocaine, 30 mg of prednisolone, 1 gram of cefazolin. Blockade is recommended at the end of surgical treatment for various lesions and purulent diseases. However, such blockades were not used to treat hand and finger infection. On the other hand, bandaging at panaritium is extremely painful, that complicates performing of stage neurectomies. We used the blockade in this formula in cases of destructive forms of hand and fingers phlegmons. The blockade was performed immediately after the surgery, as well as before the bandagings from 1 to 4 days of the postoperative period. The treatment of 115 patients was carried out in this way.

With acquisition of portable ozonizers and with receiving of positive experience in the treatment of phlegmons of various localizations we began to introduce ozone blockades into practice as well. The devitalized tissues were impregnated with an ozone-oxygen mixture with an ozone concentration of 1.0-3.0 mg/l. In total 108 patients were treated with ozonated solutions. The results were estimated clinically (including the degree of edema, hyperemia, the character of the wound fluid), with the help of microbiological cultures, cytogram analysis, comparison of the wound pH level, assessment of the index of double plasma bounds.

The clinical efficacy of the offered approach has been achieved with the combination of regional anti-inflammatory and ozone blockades. With the use of anti-inflammatory blockades, the hospitalization terms reduced by 3.2 days and at ozonization of the injectable solutions by 3.6 days. In the previous years, it was necessary to amputate a finger or a phalanx quite often (on average 2.1%). After the introduction of

blockades, the cases of finger amputation became single and did not exceed 1% per year. When comparing the obtained signatures from the wound surface the regenerative type of the cytogram started to prevail. The pH level of the wound fluid the next day after the surgical treatment became normal. These indicators are explained by the fact that ozone contributed to a reduction of tissue hypoxia and restoration of metabolic processes with pH level correction in the affected tissues on the multivalent basement, blocking the morphological zones of ischemia and toxemia. Thus, the complete surgical treatment, antibacterial therapy, and combination of adjuvant blockades have reduced the number of complications, repeated surgeries, and treatment periods. The function of hands and fingers in all patients is preserved.

Keywords: hand infection, secondary necroses, regional anti-inflammatory and ozone blockades

EVALUATION OF BIOCHEMICAL PARAMETERS OF BLOOD SERUM AFTER SUBCHRONIC EXPOSURE OF SINGLET OXYGEN IN THE EXPERIMENT

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Introduction

Singlet-oxygen therapy is used as an alternative method of stimulating of antioxidant protection. However, singlet oxygen (SO) has a strong oxidizing potential.

The aim of the study was a biochemical assessment of subchronic inhalation of SO in the experiment.

Materials and methods

We formed 3 groups from 19 Wistar rats in the experiment: 1-healthy animals (control), 2 and 3 – rats were exposed to a gas mixture containing SO. SO received using the “Airnergy” (Germany) with the capacity of the generator 100%. The procedure was repeated daily for 30 days, with a duration of 10 minutes. Rats of 2 groups were removed from the experiment on the 30th day, animals of 3 groups-on the 60th day (30 days after the course of action) by decapitation under anesthesia (zoletil + xyl). The determination of biochemical parameters of blood serum (glucose, total bilirubin, urea, creatinine, total protein, albumin, aspartate aminotransferase, alanine aminotransferase) was carried out on an automatic analyzer ILAB 650 (Italy, USA, Japan). Statistical analysis was carried out using Statistica 6 (StatSoft, Inc.). The comparison of independent variables was performed by the Mann-Whitney U-criterion.

Results and discussion

The decrease of glucose content in blood serum in rats of the 2nd group was revealed. The decrease in glucose concentration is probably due to its active use in glycolysis. In the 3rd group the glucose content was higher than in the control and the second group. Gluconeogenesis and tricarboxylic acid cycle influence on glucose level. The activity of aspartate aminotransferase (ASAT) increased in the 2nd and 3rd group of animals in comparison with control. ASAT activity was higher in the 3rd group than in the 2nd group. This indicates a decrease in adaptive mechanisms. The albumin level in the 2nd and 3rd group was lower than in the control.

The content of urea was higher in animals of the 2nd group in comparison with control on the background of stable creatinine level. The increase in urea concentration due to the decrease in albumin indicates the active use of amino acids in the process of gluconeogenesis. The level of urea in the 3rd group was lower than in the 2nd group and corresponded to the control range. There was a decrease in the total protein

content in group 3 in comparison with the indicator of group 2. This could reduce the concentration of the final product of protein metabolism-urea.

There were no statistically significant differences in the indicators of total protein, creatinine, total bilirubin and alanine aminotransferase activity in rats of the second group compared to the control.

Conclusion

Thus, the activation of energetic metabolism as a result of chronic exposure of singlet oxygen was revealed. The decrease in protein metabolism (total protein, albumin, urea) and the increase in glucose concentration were established during the subchronic use of singlet oxygen.

Key words: singlet oxygen, biochemical parameters, serum

MEDICAL OZONE PROMOTES NRF2 PHOSPHORYLATION REDUCING OXIDATIVE STRESS AND PROINFLAMMATORY CYTOKINES IN MULTIPLE SCLEROSIS PATIENTS

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Introduction

Oxidative stress and inflammation play key roles in the pathogenesis of Multiple sclerosis (MS). Different drugs have been used in the clinical practice, however, there is not a completely effective treatment. Due to its potential therapeutic action, medical ozone represents a promising approach for neurodegenerative disorders. The **aim of the present study** was to address the role of ozone therapy on the cellular redox state in MS patients.

Material and methods

Ozone (20 µg/mL) was administered three times per week during a month by rectal insufflation. The effect of ozone therapy on biomarkers of oxidative stress and inflammation was addressed by spectrophotometric and ELISA techniques. Furthermore, we investigated the action of ozone on CK2 expression and Nrf2 phosphorylation by western blotting analysis.

Results

Medical ozone significantly improved ($p < 0.05$) the activity of antioxidant enzymes and increased the levels of cellular reduced glutathione. In accordance, a significant reduction ($p < 0.05$) of oxidative damage on lipids and proteins was observed in ozone-treated patients. As well, the levels of proinflammatory cytokines TNF and IL-1 α were lower after ozone treatment. Ozone therapy incremented the CK2 expression together with Nrf2 phosphorylation in mononuclear cells of MS patients.

Conclusion

These findings suggest that ozone's antioxidant and anti-inflammatory effects might be partially associated with an induction of Nrf2 phosphorylation and activation. These results provide new insights on the molecular

events modulated by ozone and pointed out ozone therapy as a potential therapeutic alternative for MS patients.

Key words: multiple sclerosis, ozone therapy, oxidative stress, proinflammatory cytokines, CK2 expression, Nrf2 phosphorylation

COLD PLASMA AS A BIOREGULATOR: BIOPHYSICAL AND PHYSIOLOGICAL ASPECTS

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Plasma medicine is one of the most modern synthetic scientific directions born at the junction of plasma physics and biomedicine. At the same time, the greatest attention of researchers is attracted by the field of plasma medicine associated with the disclosure of biological and sanogenetic effects of cold plasma. Currently cold plasma is commonly understood as an ionized gas of different composition, cooled to physiological temperature (30-40°C). In recent decades a number of potentially beneficial effects for medical purposes have been experimentally and clinically demonstrated for this factor. First of all, antibacterial activity associated with the direct damaging effect of cold plasma on the cell wall of microorganisms is well studied and described.

Special interest, in our opinion, has the bioregulatory properties of cold plasma with respect to the functional and metabolic parameters of living systems. So, the regenerative activity of the factor and its ability to inhibit tumor growth was previously demonstrated. Of course, these empirical facts require multi-stage verification, but even the existence of such data indicates the presence of secondary, indirect biological effects of cold plasma, which allows us to consider it as a potential bioregulator.

In our previous studies, performed both *in vitro* and *in vivo*, the existence of secondary bioregulatory properties in cold plasma was shown and verified. In particular, even a short treatment (1-3 min.) of blood samples with a flow of helium cold plasma led to the formation of a biosystem response (according to metabolic and physico-chemical criteria), and it is important to emphasize the dose dependence of the detected shifts. It should also be noted that the nature of the changes was significantly different from the "pattern" of the response to the similar effect of the non-ionized helium flow from the same source. Interestingly, the reaction of isolated blood from the body as a simple model biosystem as a whole human blood turned out to be co-directed by shifts in the same blood parameters of rats subjected to short-term (1-2 min.) exposure to cold plasma on the pre-epilated areas of the back. As with *in vitro* experiments, in this case a moderate antioxidant effect of the factor and its positive effect on the intermediate link of energy metabolism are shown. In addition, a modulating effect of helium cold plasma on the parameters of systemic and local hemodynamics was demonstrated.

Thus, plasma biomedicine is a promising, dynamically developing direction, integrating biophysical and biomedical approaches and capable of forming new medical technologies, potentially useful for the correction of various diseases, pathological conditions, injuries and burns.

Key words: cold plasma, biological effects, antibacterial activity, metabolism, hemodynamics

MODIFICATION OF BLOOD PHYSICAL AND CHEMICAL PROPERTIES UNDER THE ACTION OF HELIUM COLD PLASMA

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The aim of the study was comparative estimation of the changes of oxidative metabolism and crystallogenic properties of blood plasma under processing with cold helium plasma and non-ionized helium flow.

Material and methods

We studied the influence of microwave-generating cold plasma on the specimens of whole human blood. The exposure time was 1 and 3 min. Before processing all blood specimens were divided into 5 portions. First portion was control (without any manipulations), second and third portion were treated with cold plasma, fourth and fifth ones were sparged with non-ionized helium flow. In all portions we estimated the parameters of oxidative metabolism and crystallogenic activity. For oxidative metabolism estimation we used Fe-induced biochemiluminescence with registration of light sum as an index of intensity of lipid peroxidation and parameter $tg2\alpha$ as a criterion of total antioxidative activity. Crystallogenic properties were studied with teziocrystalloscopy method. For description of crystalloscopic facia we used own semi-quantitative criterias.

Results

It was stated that cold helium plasma and non-ionized helium modified these parameters under blood processing in vitro. For cold helium plasma this effect was realized by stimulation of antioxidant activity and crystallogenic properties of blood plasma. In opposite, non-ionized helium flow had prooxidant effect and demonstrated the inhibition of biological fluid crystallization.

Conclusion

Our data shown that most optimal time for blood processing with cold plasma is 1 min.

Key words: cold plasma, biological effects, blood plasma, oxidative metabolism, crystallogenic properties

BIOCRISTALLOMICS IN PERSONIFICATION AND MONITORING OF TREATMENT AT PATIENTS WITH BURNS

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The **purpose of this study** was to evaluate the informative value of the study of crystallogenic properties of blood serum in the monitoring of the effectiveness of ozone therapy in patients with burn disease.

Material and methods

The study included 30 patients with thermal injury, divided into 2 equal groups. Patients of first (main) group received a standard treatment protocol in accordance with federal management of burn patients, which was supplemented with a course of systemic ozone therapy. This course, starting with 3-4 days post-burn period, consisted of 10 daily procedures intravenous administration of ozonized physiological solution (200 ml; ozone concentration of 3000 mcg/l). Patients of the second group (comparison group) received treatment only in the framework of the approved federal standard. Before and after the course of ozone therapy the patients had received blood samples with subsequent separation of the serum. Evaluation of crystallogenic properties of the blood serum was performed by the method of teziocrystalloscopy.

Results

In the process of complex treatment, representatives of both groups showed a clear tendency to normalize own and initiated crystallization of blood serum, but the severity of these shifts was different. Thus, in patients of the comparison group, there was a moderate increase in crystallizability and structure index in the crystallographic facies of blood serum ($p < 0.05$). In addition, an increase in the correctness of the latter structure was recorded, which was realized in the form of a decrease in the facia destruction degree ($p < 0.05$). Also, there was a slight expansion of the marginal zone of serum crystalloscopic facies (by 13%; $p < 0,1$). In patients with burns, who additionally received an ozonated physiological solution infusions, a more pronounced tendency to normalize the crystallogenic properties of blood serum was registered. In particular, the appearance of not only single-crystalline, but also small dendritic elements was found in the crystallographic facies of the biological liquid, which caused a significant increase in crystallizability and structure index. This level of the parameter is statistically significantly higher than the figures for the first observation point ($p < 0.05$). It should be noted that in this case, the values of both indicators significantly

exceed those found for patients of the comparison group ($p < 0.05$). The degree of destruction of facies in patients of the main group is reduced more clearly than the patients of the comparison group ($p < 0.05$). The size of the marginal zone of the micro product also increases significantly (by 29%; $p < 0.05$).

Conclusion

It is established that the addition of a standard protocol of management of severe burned patients to the course of systemic ozone therapy (the introduction of ozonized physiological solution) contributes to the optimization of physico-chemical properties and, therefore, the component composition of blood serum. In addition, the results allow us to consider the study of crystallogenic properties of this biosubstrate as a method of evaluating the efficiency of correction of metabolic disturbances that occur during the development of the victims of burn disease.

Key words: burns, metabolic rehabilitation, crystallization, blood serum, biocrystallogics

METABOLIC ESTIMATION OF SUBCHRONIC TOXICITY OF NITRIC OXIDE

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The **aim of the study** was to assess the effect of a long course of inhalations NO (30 days) on the parameters of oxidative metabolism of the blood.

Material and methods

The experiment included 30 male Wistar rats. Three groups of animals were formed: a control group (n=10), including animals that did not perform manipulations; and two main groups (n=10 in each), animals that received inhalations of a gas mixture containing an increased concentration of oxygen (93±3%) or nitric oxide (concentration – 20 ppm), respectively. Inhalations were carried out daily for 30 days, their duration was 10 minutes. The state of oxidative and energy metabolism of rat blood in the dynamics of influence was determined. For oxidative metabolism estimation in blood plasma and erythrocytes we used Fe-induced biochemiluminescence with registration of light sum as an index of intensity of lipid peroxidation and parameter tg2α as a criterion of total antioxidative activity. Level of malonic dialdehyde was tested with V.G. Sidorkin and I.A. Chuloshnikova (1993) method. Activity of superoxide dismutase was studied with T.V. Sirota (1999) method.

Results

It is established that inhalations of oxygen and nitric oxide in long-term use (30 days) have a different effect on the parameters of oxidative and energy metabolism of rat blood. The antioxidant effect of moderate stimulation of lipoperoxidation processes and marked increase of antioxidant activity of blood plasma was noted. At the same time, a more pronounced increase in the total activity of plasma antioxidants was found for oxygen inhalations, whereas the use of NO significant component of the metabolic response is the strengthening of the catalytic properties of erythrocyte superoxide dismutase. According to the parameters of energy metabolism, a more favorable reaction was noted during the inhalation of oxygen, and when exposed to NO, a moderate activation of the enzyme reverse reaction was registered.

Key words: nitric oxide, inhalation, subchronic toxicity

THE INFLUENCE OF INHALATIONS OF REACTIVE OXYGEN SPECIES ON MICROCIRCULATION

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The **aim of the work** was a comparative assessment of the influence of inhalations of ozone and singlet oxygen on the intensity and regulatory mechanisms of microcirculation of rats.

Material and methods

The first group of rats (n=10) was intact. Animals of the second to fifth groups (n=10 in each group) received daily inhalations for 10 days. Inhalations of singlet oxygen gas mixture were carried out in the second group, using 100% capacity of the generator "Airnergy Professional plus" (Germany), in the third – dry ozone-oxygen mixture (ozone concentration – 60 µg/l), in the fourth – moistened ozone, in the fifth – ozone-oxygen mixture passed through a layer of olive oil. The duration of all procedures is 10 minutes. The state of microcirculation was assessed by laser Doppler flowmetry at "LAKK-M" device (Russia). The intensity of blood flow through microvessels was monitored by the index of microcirculation, the activity of regulatory mechanisms – by the level of the relevant components (endothelial, neurogenic, myogenic, cardiac and respiratory), and the nature of the inclusion of shunting paths - by the value of the shunting index.

Results

It was revealed that only singlet oxygen inhalations contribute to the increase of blood flow intensity along the microcirculatory bed (by 14%; p<0.05). The remaining effects provided a decrease in the microcirculation index, and when using moistened ozone and oil ozonides, this effect was moderate (by 25 and 16%, respectively; p<0.05). On the contrary, inhalations of dry ozone-oxygen mixture led to severe depression of microcirculation (at 66%; p<0.05).

When using a dry ozone-oxygen mixture, the inhibition of all "internal" regulatory mechanisms (endothelial, neurogenic and myogenic) in combination with stimulation of "external" influences (respiratory and cardiac components) was observed. On the contrary, inhalations of singlet oxygen gas mixture provide primary activation of endothelial and neurogenic mechanisms, while maintaining the rest at a level close to intact. The peculiarity of the action of moistened ozone is the stimulation of the myogenic component of regulation, and oil ozonides – the maximum safety of all mechanisms. After a course of inhalations of singlet oxygen and moistened ozone, a decrease in shunting was recorded (by 20 and 25%, respectively; p<0.05 for both cases) and its approach to 1 rel. un.

Conclusion

Thus, the optimal reaction of local hemodynamics on the inhalation of the gas stream from the generator of singlet oxygen and moistened ozone-oxygen mixture compared to dry ozone and oil ozonide. A special feature of the action of singlet oxygen is the stimulation intensity of the microcirculation by the activation of the "internal" regulatory mechanisms.

Key words: singlet oxygen, ozone, oil ozonides, inhalation, hemodynamics, microcirculation

DOSE-DEPENDENT EFFECT OF SINGLET OXYGEN OF SOME PHYSICAL AND CHEMICAL PARAMETERS OF THE BLOOD

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Introduction

The **aim of the work** was to assess the dynamics of physical and chemical parameters of blood in its treatment with singlet oxygen in vitro.

Material and methods

Samples of whole preserved blood from healthy donors (n=10) were used for the study. Each sample was divided into 5 portions of 5 ml and placed in clean dry test tubes. The first portion was control, it had no impact. The second portion was treated by bubbling with an oxygen-ozone mixture (ozone concentration – 500 µg/l), the third - pure oxygen, the fourth and fifth – a gas mixture containing singlet oxygen (at a generator power of 50 and 100%, respectively). The duration of the bubbling was 3min. in all cases. Synthesis of ozone-oxygen mixture was carried out with the help of ozonator "Medozons-BM" (Russia). The air stream containing singlet oxygen, obtained with the use of "Airnergy" device (Germany). Exposure after processing was 3 minutes. The acid-base equilibrium and partial pressure of blood gases were determined using an automatic analyzer "ABL-77". The results were processed using Statistica 6.0.

Results

Taking into account the fact that as a result of the studied effects oxygen-containing gas mixtures are introduced into the blood, after their completion, significant shifts in the gas composition of the blood were observed. In particular, all considered factors provided reduction of partial pressure of carbon dioxide practically on 50% in relation to initial level ($p<0,05$), however only pure oxygen and oxygen-ozone mix considerably raised partial pressure of oxygen in blood more than in 2 times in comparison with control values ($p<0,05$).

Under the influence of the reactive oxygen species the parameters of acid-base balance of the blood are changed. Thus, all the studied factors lead to moderate latching of blood samples (0.13; 0.20; 0.18; 0.18 and 0.20 rel. units in the processing of oxygen, oxygen-ozone mixture and singlet oxygen for 50 and 100% power, respectively ($p<0.05$ for all cases). The mechanism of these shifts may be associated with the generation of hydroxide ions in the degradation of reactive oxygen species in the biological fluid, as well as with a decrease in the concentration of dissolved carbon dioxide. The last tendency is equally visible for all studied influences both on the general level of this parameter, and concentration of bicarbonate in blood plasma (over 70-75% from the original values ($p<0.05$)).

Conclusion

In general, it is found that the treatment of blood with singlet oxygen in vitro optimizes the gas composition and parameters of the acid-base balance of the biological fluid.

Key words: singlet oxygen, blood, acid-base equilibrium, partial pressure of blood gases

EVALUATION OF THE EFFECT OF OZONE THERAPY ON THE OSSEOINTEGRATION OF DENTAL IMPLANTS

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The success of dental implants has been attributed to osseointegration or direct contact of the implant surface and bone. The purpose of this study was to evaluate the effect of ozone therapy on the osseointegration of dental implants inserted in experimental animals as well as in patients received implant supported mandibular overdentures. Eight New Zealand white mature male rabbits were received 16 dental implants inserted in both tibiae, as well as in eight completely edentulous male patients were received mandibular implant overdentures supported by two implants in the canine area. The implants in the right tibiae and in the right canine regions (control group) were inserted by the conventional method. The implants in the left tibiae and left canine regions (test group) were inserted by the conventional method nevertheless; irrigation with ozonized water of 25µg/mL concentration was applied during the osteotomy procedure. Ozone gas of 60 µg/mL concentration was applied topically in the performed osteotomy sites before the insertion of the implants. After implantation two rabbits were euthanized after two, four, six and eight weeks. The tibiae were dissected from each rabbit and specimens were prepared for histologically and histomorphometric analysis. Clinical and radiographic evaluations were carried out for every patient at the time of loading the prosthesis, then three, six and up to 12 months respectively. Within the limits of this study, the results suggest that the success of osseointegration of the dental implants was achieved in both groups. Moreover, the ozonized group may reveal acceleration of osseointegration, also it may influence bone density in peri-implant interface.

Key words: dental implants, ozone therapy, osseointegration, experiment

POSSIBLE MAJOR AUTOHEMOTHERAPY (MAHT) COMPLICATIONS IN THE PRACTICE OF OZONE THERAPY DEPARTMENT OF THE SANATORIUM

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We would like to tell the specialists, who practised Major Autohemotherapy, about possible complications which patients can have, according to practice of the sanatorium's Ozone Therapy Department.

Major Autohemotherapy (MAHT) is a systemic treatment method with the help of medical ozone. At the present time MAHT is an essential method of systemic ozone therapy included in the Madrid Declaration on ozone therapy by the International Scientific Committee of ozone therapy on the 4th of June in 2010 (ISCO3). MAHT means treatment of a patient's blood using a gaseous ozone-oxygen mixture and reinfusion of the blood.

There are exceedingly rare cases of MAHT complications in literature (Jacobs, Marie Theres, 1982).

MAHT procedure. Our specialists use blood bags (a South Korean product), which are with a volume of 250 ml, for blood drawing. The blood bags consist of 35 ml of Glugicirum (anticoagulant-conservative). In a therapeutic context a patient is in prone position and a specialist, following aseptic and antiseptic techniques, draws 100 ml of the patient's blood which gets into the Hemocon and are mixed with the same volume of a gaseous ozone-oxygen mixture. Blood reinfusion gets into the patient's vein through the same needle. A resend rate of ozone blood depends on a distance between the blood bags and the patient. This procedure takes about 10 minutes. At the end of the procedure the specialist applies a tight sterile dressing the patient has to wear for an hour after the procedure.

Over 18000 procedures have been conducted at the sanatorium's Ozone Therapy Department for 11 years (2007-2017). About 6500 people have been treated (a course vary between 1-2 and 7-8 procedures). Average and high doses have been used (from 0,5 to 5 ml per procedure).

Patients can often have minor local injuries after using a puncture needle by a specialist. Patients can manage or reduce a risk of having these injuries if they follow recommendations on wearing and using self-adhesive bandages.

Patients can rarely have transient losses of consciousness or light-headednesses. It is necessary to have a patient's medical history such as blood drawing and procedure tolerance, check a patient's psychoemotional state correctly, control a patient's blood pressure before the procedure to avoid these problems.

10 cases of allergic reactions have been registered (0,15 per cent out of the treated patients or one reaction out of 1800 procedures) at the Ozone Therapy Department for 11 years. Only once the procedure has been

stopped because of a case of the patient's repeated sneezing during the first MAHT. There has been one case of neck edema but the patient has complicated anamnesis for allergic events (during the third procedure of the first course). There have been 3 cases of urticaria after refresher MAHT courses and 5 cases of anaphylactic reactions (4 patients in the refresher course of treatment and one patient during the first procedure) during autoblood reinfusion.

Our specialists have taken standard measures without using adrenergic agonists to avoid an anaphylactic shock after these complications. Thus, allergic reactions are possible during MAHT. Patients can have some reactions such as urticaria and edema several hours later after the procedure. Perhaps these reactions are not the results of MAHT. The complications during the therapy can be caused by a patient's reaction to Glugicirum. A cough during the procedure is a serious symptom which can suggest an anaphylaxis reaction.

Key words: major autohemotherapy, complications, an anaphylaxis reaction

ASSESSMENT OF THE STATE OF MICROCIRCULATION UNDER THE INFLUENCE OF EXOGENOUS NITRIC OXIDE ON THE BACKGROUND OF THERMAL INJURY

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Introduction

In the early period of the combined thermoinsulation injury in the problems of comprehensive treatment along with activities for the fight against pain, compensation of the deficits of the volume of circulating plasma, the elimination of violations of hemorheology, increasing neutralization of endotoxemia, the maintenance and stimulation of excretory functions of the body include the treatment of circulatory and transport dysfunction.

Materials and methods

The experiment was performed on 18 Wistar rats weighing 180-250 g., divided into 2 equal groups. In the first group (n=9) and control animals caused a combined thermal injury, including contact burns on 20% of p. T. animal and thermoinsulation injury, the second (n=9) – experimental – thermal injury is followed using the apparatus "PLASON" (Moscow), in the regime of NO-therapy at minimum flow rate and distance from the wound 1.5 – 2 cm for 2 min.

The state of microcirculation was assessed using laser Doppler flowmetry (LDF) on the device "LAKK-M" (NPO "Lazma", Moscow). The indicator of microcirculation (PM), as well as active (endothelial, neurogenic, myogenic) and passive (respiratory, cardiac) regulatory factors were considered. The state of microcirculation of animals was registered initially, as well as on the 7th and 14th days from the beginning of the experiment. Analysis and statistical processing of the obtained data was carried out using Statistica 6.1.

Results and discussion

It was found that the microcirculation index in the control group decreased at all stages: on the 7th day the decrease was 39%, and on the 14th day-46% ($p < 0.05$). It is shown that on the 7th day in the experimental group the indicator normalizes relative to control values in the same period, with the subsequent intensification up to 223% of normal values. The reaction of regulatory factors proved to be multidirectional. Thus, in the control group, the growth of the respiratory component was revealed by 9% compared to the 7th, and 68% compared to the 14th day relative to the starting values. In the experimental group, on the contrary, a decrease of 36% and 62% ($p < 0.05$), respectively, relative to the norm was observed in the same period. Such a reaction can be explained by the fact that the additional application of nitric oxide increases venous outflow.

Conclusion

Thus, it is shown that external application of the source of exogenous nitric oxide promotes intensification of microcirculation at the combined thermal trauma, to a greater extent due to passive factors of regulation, mainly respiratory.

Key words: microcirculation, thermal trauma, nitric oxide

OZOTENS-THERAPY OF DORSALGIAS AND ARTRALGIAS

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It is known that about 90% of diseases is associating with pain syndrome are treated by specialists from primary medical care (Scoping Document for WHO Guidelines, 2012). Neurologists stated that most important cause of pain syndrome in dorsopatia is degenerative processes in vertebrae as a component of osteochondrosis pathogenesis. Other serious problem of modern orthopedics are arthralgias, which are combined with posttraumatic osteochondrosis of крупных суставов.

The **aim of this work was** the study of possibilities of electromyostimulation (EMS) with transcutaneous electrophoresis of cream with reactive oxygen species in correction of pain syndrome at patients with dorsoalgias and arthralgias.

Material and methods

We evaluated 43 patients with lumbar dorsopathy or arthralgia of the knee (62-73 years old), divided into 2 groups. Patients of control group (n=20) got standard analgesics, vascular and antioxidant therapy, vitamins. Physiotherapy for these patients included EMS on some zones (thoracic and lumbar paraarticular zones of the spine, paraarticular zones near the knee). Patients of main group (n=23) additionally got a innovative manipulation – OZOTENS-Therapy, which based on EMS, but it modified with electrophoresis of creams with high dose of reactive oxygen species (6000 mg O₂/kg). Procedures were performed on low-frequency electro- impulse massager “MN8002 COMBO”. All the patients were tested clinically (estimation of pain syndrome), instrumentally (computer tomography and magnetic resonance tomography of lumbar region of the spine or knee zone; study of central and peripheral hemodynamics, microcirculation state; spirometry) and laboratory (by parameters of blood oxidative and energy metabolism, acid-base balance indexes). Statistic calculations were provided by standard algorithms with Statistica 6.1 for Windows.

Results

Our studies allow to demonstrate significant clinical improvement in 19 patient of main group (88,6%) and 15 patients of control group (75%). This dynamics is characterized by clear decreasing of pain syndrome and full restoration of motions in lumbar region of the back. In addition, OZOTENS–therapy caused the stimulation of microcirculation (at 1.6-3.3 times to initial level, p<0.05) with prevalence of parasympathetic influence, increasing of myocardium work and power and respiratory support. We laboratory fixed that OZOTENS–therapy led to intensification of free radical processes with prevalent activation of antioxidant reserves and optimization of energy metabolism.

Conclusion

In whole, our study shown that sanogenic effects of EMS/OZOTENS-therapy include myostimulation, myorelaxation, electrolysis and bioactivating action on needed zones. Transcutaneous transport of reactive oxygen soecies from cream occurs by eletroporation of biological structures.

Key words: dorsalgia, artralgia, ozone-contianing cream, electromyostimulation, elecporation, electrophoresis

NEW PERIOPERATIVE NITRIC OXIDE INHALATION TECHNIQUE FOR PATIENTS WITH HIGH PULMONARY HYPERTENSION DURING OPERATIONS WITH CARDIOPULMONARY BYPASS

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The **aim of the study** was to evaluate the effectiveness of a new technique of permanent inhaled NO therapy on lungs protection during cardiac valve operations with cardiopulmonary bypass (CPB) in patients with high pulmonary hypertension.

Material and methods

The study included 47 patients of both sexes aged 25 to 76 years (mean age 58.9 ± 1.9) who underwent heart valve operations or combined interventions. The mean pulmonary artery pressure was 42.2 ± 1.5 mm Hg. All patients gave informed consent, and the study was approved by the ethics committee of the institution. Patients were divided into two groups: the first (27 patients) - control, where no specific measures were taken to protect the lungs; the second (20 patients) - patients received NO inhalation from transferring to artificial ventilation during the whole operation, pulmonary artery perfusion and lungs ventilation with NO during CPB were performed. A comparative evaluation was carried out based on the dynamics of the lungs functional parameters.

Results

NO inhalation before CPB was carried out at a concentration of 17.8 ± 0.71 ppm, during CPB 17.4 ± 0.67 ppm, in the post- CPB period - 18.5 ± 0.60 ppm. The content of NO₂ was: before CPB - 1.40 ± 0.06 ppm, during CPB - 1.40 ± 0.06 ppm, after CPB - 1.60 ± 0.12 ppm. The content of methaemoglobin in all patients did not exceed 1.5%. The administration of inhaled NO therapy was accompanied by a significantly lower alveolar-arterial oxygen difference during operation, a significantly higher oxygenation index from the onset of NO inhalation, and a higher level at the end of the surgery, significantly lower intrapulmonary shunting after CPB and preservation initial values of pulmonary compliance at all stages of the operation.

Conclusion

The application of the new perioperative nitrous oxide inhalation technique effectively preserved the oxygenating function of the lungs, pulmonary compliance, decreased the intrapulmonary shunting of the blood after CPB in high risk patients with severe pulmonary hypertension.

Key words: inhalation therapy, nitric oxide, lungs protection, operations with cardiopulmonary bypass

CLINICAL INVESTIGATION OF EFFICACY NITRIC OXIDE PRECONDITIONING ON MYOCARDIAL PROTECTION DURING CARDIAC VALVES OPERATIONS WITH CARDIOPULMONARY BYPASS

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The aim of the study was to evaluate the effect of intraoperative NO inhalation therapy on the effectiveness of myocardial protection during heart valves surgery in conditions of cardiopulmonary bypass (CPB) and combined pharmacological cardioplegia with crystalloid solutions (Custadiol).

Material and methods

The study included 60 patients of both sexes aged 33 to 75 years (mean age 57.6 ± 0.9) who underwent heart valve operations or combined interventions. The operations were performed under conditions of normothermic CPB and combined cardioplegia («Custadiol»). All patients were randomized into two groups: the first (30 patients) - control, where as the main method of protecting the myocardium was cardioplegia based on "Custadiol"; the second (30 patients) - additionally, all patients underwent inhalation therapy with nitric oxide from the moment of transferring to artificial ventilation throughout the operation. As a generator of nitric oxide, the «Tianox» apparatus (Russia) was used, nitrogen oxide ($0.3-0.4$ l / min) was supplied to the inspiratory path in a stream of pure oxygen ($1.5-2$ l / min) at a concentration of 20-25 ppm. Clinical evaluation was carried out on the basis of clinical indices (the rate of spontaneous cardiac recovery, post-ischemic heart rhythm disturbances, dosages of inotropic drugs) and the dynamics of the marker of myocardial damage (troponin I) in the postoperative period (after surgery, 12, 24 and 48 hours).

Results

The spontaneous cardiac recovery after ischemia was more often observed in patients in the 2nd group (56.6%), in the 1st group - in 40%, the more rare development of postischemic rhythm disturbances (13.3%, in 1 st group - 26,7%). There were no significant differences in the dosages of inotropic drugs. Dynamics of troponin I concentration: baseline levels did not have significant differences. At the end of the operation, the level of this indicator significantly increased in both groups of patients: in the first group - from 0.010 ± 0.008 to 2.620 ± 0.206 ng / ml, in the second group with 0.010 ± 0.001 to 1.180 ± 0.149 ng / ml, it should be noted that the level of the marker at this stage in patients of the 2 nd group was significantly lower (2 times) than in the patients of the 1 st group. Twelve hours after the operation, both groups showed a significant increase in the concentration of troponin I (in the first group, from $2,620 \pm 0,206$ to $3,410 \pm 0,407$ ng / ml, in the second group from $1,180 \pm 0,149$ to $2,020 \pm 0,167$ ng / ml). However, the level of the marker in the patients of the 2

nd group is also significantly lower than in the patients of the 1 st group. Decrease in the concentration of troponin I started 24 hours after the operation, which continued to the end of 2 (after 48 hours) days. At these stages, there were no significant differences.

Conclusion

Clinical evidence suggests a protective effect of inhaled nitric oxide on the myocardium. The effect of pharmacological preconditioning manifested itself in an increase in the frequency of independent cardiac recovery after ischemia, a decrease in the rate of postischemic rhythm disturbances and lower levels of troponin I over the next 12 hours of the postoperative period. Nevertheless, further clinical studies are needed to confirm the effect of this technology.

Key words: inhalation therapy, nitric oxide, pharmacological preconditioning, myocardial protection

OZONUCLEOLYSIS IN BRACHALGIA

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Background and purpose

Direct injection of Oxygen-Ozone in the cervical discs has proved to be the effective alternative to surgery in patients with cervical disc herniation in many countries around the world. We report our experience with ozonucleolysis with patients effected by pain in cervical region (Brachalgia) due to disc herniation including post operative recurrence or disc prolapse.

Methods

4000 patients were treated with single session of Oxygen Ozone therapy from 2005-2017. All the patients had CT or MRI evidence of cervical disc prolapse with clinical signs of cervical nerve root compression. The procedure was performed under angio fluoroscopy using 22/23 G spinal needle with out any form of anesthesia. All the patients received intra discal injection of Oxygen Ozone mixture at a ozone concentration 30 ugm/ml. Among 4000 patients 3000 were males and 1000 were females between the age of 20-70 yrs. Therapeutic out come was assessed 5 months after treatment by using modified MacNab method.

Results

A satisfactory therapeutic outcome was obtained. 60% of the patients showed complete recovery with resolution of symptoms. 20% of the patients complained of occasional episodic neck pain and arms pain with no limitation of occupational activity. 5% of cases showed insufficient improvement. 5% of cases had no improvement and went for surgery. 10% of cases never turned up after the first visit.

Conclusion

Intradiscal and periganglionic injection of Ozone for herniated cervical disc has revolutionized percutaneous approach to nerve root diseases making it safer, cheaper and easier to repeat than treatments currently in use.

Key words: ozonucleolysis, brachalgia, disc herniation, ozone

IV OZONATED MARINE PLASMA AS A KEY ELEMENT IN THE RESTORATION AND REGENERATION OF FUNCTIONS AND / OR TISSUES

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This paper is intended to promote the dissemination, research and use of ozonated marine plasma and to provide ozone therapy with a broader field of application without risks and with better results.

Patients were randomly selected according to case complexity and chronicity.

CASE 1: man, 40 years old. Chronic rheumatoid arthritis, thirty years of evolution, aggressive and not cured or alleviated in any way, accompanied by severe fibromyalgia and subcutaneous nodules.

CASE 2: woman, 50 years old. Multiple abscesses in different parts of the body, eight years of evolution without results.

CASE 3: man, 67 years old. Abscess in left foot, of unknown etiology, that finally developed sepsis.

In all three cases IV ozonated marine plasma was used in the form of localized ozone injections; hypobaric capsule; medical equipment ozonator OXI3.

Marine plasma provides the possibility of achieving results quickly and optimally, without risks and with the following advantages: balance of the interior milieu, alkalinity, oxygen saturation, hydration, nutrition, original DNA (carrying the information regarding the origins of cellular life), organic elements and ionic substitution. It possesses all the chemical elements of the periodic table.

Limitations: it is highly important to administer in small doses given the power of the results (not yet measured, but proven in each case); increase in frequency of Jarisch-Herxheimer reaction after sessions, it is easy to recover from and there are no complications when patients are properly informed.

Key words: marine plasma, restoration, regeneration, alkalinity, oxygen saturation, ozonides, nutrition

PIE DIABÉTICO, TRATAMIENTO MÉDICO MULTIDISCIPLINARIO Y OZONOTERAPIA, UNA ALTERNATIVA MÁS

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Diabetic foot ulcers in Diabetes patients are consider a medical emergency and a public health problem, being the mean cause of hospital incomes and amputation. Is consider a multifactorial disease in which are involved venous insufficiency, motor and sensitive peripheric neuropathy, immunosuppression, oxidative stress, post-translational modifications developing claudication, dumbness in feet, anhidrosis, hyperkeratosis, helomas, dryness, no perceptible cutaneous loss integrity leading to ulcers, infections and gangrene. A multidisciplinary approach is recommended to deal with metabolic disorder, mobility, ulcer healing. Our proposal is to include ozone therapy as medical treatment for diabetic foot ulcers and diabetes. In the four cases that we presented with chronic infected diabetic foot ulcers diagnosis, we proposed an eight weeks multidisciplinary treatment included, diet, hygienic and general indications, pharmacological treatment for diabetes and venous insufficiency, physiotherapy and parenteral (ozonized saline solution) , and local ozonotherapy (ozonized olive oil and hermetic boot) in which we observed at the end of the treatment a glycemc low down at normal levels, a total ulcer healing and a recover of the claudication symptoms.

Key words: diabetes, foot ulcer, multidisciplinary approach, ozone therapy

OZON AS EFFECTIVE COMPONENT OF COMBINED TECHNOLOGY OF BONE GRAFTS STERILIZATION

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Introduction

One of the most common technologies for sterilizing bioimplants today is radiation treatment with γ -radiation or a fast-electron flux. In Russia, a dose of 25 kGy is taken as the "standard" dose of absorption, which determines the effectiveness of suppressing various pathogens. This value is consistent with the recommendations of the International Atomic Energy Agency (IAEA), but in some countries there are other standards (30 kGy and even more). At the same time, it is known that, the higher the dose of absorption - the stronger the radiation sterilization affects the change in the properties of bioimplants. The purpose of this study is to reduce the dose of radiation exposure through the development of a combined technology for sterilization of bone implants based on the combination of ozone and radiation treatment in a single production process.

Material and methods

As an experimental bone-plastic material, fragments of the bull's femoral diaphysis were used, from which samples of rectangular shape with dimensions of 6x8x18 mm were mechanically cut out. In the initial state, all samples were contaminated with a mixed microflora. The sample groups were separately or sequentially subjected to an ozone-air mixture and radiation treatment. The control of sterility was carried out during microbiological studies with the use of a thioglycolic medium (for the presence of aerobic and anaerobic bacteria) and the Saburo medium (for the presence of microscopic fungi).

Results and discussion

The conducted studies showed that control samples, as well as samples subjected only to ozone or only radiation treatment did not satisfy the required degree of sterility and showed the presence of dissemination on 3-14 days at least on one of the culture media. Complete sterility in both culture media after 14 days of the experiment was demonstrated only by the samples subjected to combined action - sequential treatment with an ozone-air mixture with a concentration of 6-8 mg / L for 10-20 min and radiation irradiation with absorbed dose values of 11, 15 and 27 kGy.

Conclusion

A new two-stage combined technology for sterilization of bone implants has been developed. At the first stage, the biomaterial is subjected to ozone treatment, at the second stage - radiation irradiation is carried out by a flow of fast electrons. Ozone exposure leads to a significant weakening of the population of pathogens and, as a consequence, to a decrease in their radioresistance, which in turn increases the efficiency of subsequent radiation treatment even at a half-lower dose of absorption. The developed technology is protected by the patent of the Russian Federation.

Key words: bone grafts, ozone, radiation sterilization, combined technology

OZONE THERAPY IN HUMAN PAPILLOMAVIRUS (HPV) INFECTION

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Study of 30 patients infected with human papillomavirus (HPV) without having spontaneous remission after one year as of the first diagnosis. The samples were collected between October 2014 and February 2017. Cervical swabs were analyzed both HPV genotyping by PCR and Reverse Line Blot, and cervical cytology by Pap testing.

The study was carried out with the objective of verify the efficacy of O2 / O3 alone and in combination with other complementary measures. The patients were divided into two groups.

a) **Group O3 + (treated with ozone plus integrative therapy):** It was treated with intravaginal ozone, ozonated saline solution, micro-immunotherapy and administration IV of GSH, Se, Zinc and VitC.

b) **Ozone group (treated exclusively with ozone):** It was administered with intravaginal ozone therapy alone. Although the number of patients does not allow a safe conclusion, the group of patients treated with ozone plus integrative therapy presented a great advantages ($p < 0,05$) compared to the group of patients treated exclusively with ozone.

Positive (negative cytology after 3 months of finishing the therapy)

- ▶ **Group O3 +** (patients treated with ozone plus integrative therapy): 16 de 20 => 80.0 %
- ▶ **Ozone group** (patients treated exclusively with ozone): 6 de 10 => 60.0 %

It was shown that the synergy of the ozone therapy with the other treatments was more effective, permanent and harmless than the application of intravaginal ozone alone.

Key words: intravaginal ozone, ozonized oil, human papilloma virus, ozonated saline solution, genotype 16, 18, condiloma acuminata, ozone therapy, womb cancer

INTRADISCAL OZONE FOR BACKACHE, A PAKISTANI EXPERIENCE

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Aim: Ozone is an upcoming remedy for most of musculoskeletal related pains specially for backache. Directly injecting Oxygen-Ozone in to the discs has proved to be the effective alternative for surgery in patients with disc herniation. We share our experience with ozonucleolysis with patients affected by lower backache and sciatica due to disc herniation including post operative recurrence.

Material and methods

Twenty two thousand patients were treated with single to multiple sessions of Oxygen Ozone therapy from January 2008 to June 2017. All the patients had MRI evidence of annulus tear/ disc prolapse with clinical signs of nerve root compression. All the patients received intra discal injection of Oxygen Ozone mixture under angiofloro with 22/23 G cheeba needle at an ozone concentration 27 Ugm/ml with peri ganglionic infiltration with depomedrol and 1% xylocaine. Males were 15600 and 7400 females between the age of 18-80 years. Therapeutic outcome was assessed 5 months after treatment by using modified MacNab method.

Results

A satisfactory therapeutic outcome was obtained. 75 % showed signs of recovery. Among them 55% of the patients showed complete recovery with resolution of symptoms. 20% of the patients complained of occasional episodic pain and with no limitation of occupational activity. 15% of cases showed insufficient improvement. 5% of cases had insufficient improvement and went for surgery. 5% of cases never turned up after the first visit.

Conclusion

Intradiscal injection of Ozone for herniated discs has revolutionized percutaneous approach to nerve root diseases making it safer, cheaper and easier to repeat than treatments currently used in Pakistan.

Key words: backache, intradiscal ozone therapy

THE USE OF A MEDICAL OZONIZER FOR THE PURPOSE OF INDUCING DNA DAMAGE FOR THE MODIFICATION OF COMET ASSAY TECHNIQUE

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Introduction

Comet assay reveals various DNA damage in individual nucleated cells and is used in laboratory studies to solve mainly fundamental ecological, toxicological, and biomonitoring problems (Sirota and Kuznetsova, 2010; Tice et al., 2000; Collins et al., 2008). However, the comet assay is not currently used as a standard analytical method in clinical laboratories, since there are some methodological difficulties that prevent its good reproducibility and accuracy (Tice et al., 2000). Most researchers use gamma radiation at specific doses as a test load on DNA in individual cells (Sirota and Kuznetsova, 2010). However, in order to ensure this approach, it is necessary to comply with a number of requirements of sanitary standards. As a result, the need to modify the comet assay technique at certain stages of its protocol remains relevant. The purpose of this work was to assess the possibility of using ozone to induce DNA damage in individual cells.

Materials and Methods

The experiments were performed with peripheral blood leukocytes of 13 non-linear intact non-native male rats (5 months of age). For each blood sample, three microscopic slides were prepared, consisting of two layers of low-melting agarose, upper of which contained individual nucleated cells. DNA damage in these cells was induced by phosphate-buffered saline (pH 7.4), through which the ozone-oxygen mixture was preliminary passed for 5 min at a gas flow rate of 0.5 L/min and ozone concentration in a mixture of 200, 400, and 900 µg/L with the help of the medical ozonizer AOT-NSK-01-"C (A-16)" TM "Teozon" (Sarov, Russia). The cells in the agarose slides were treated for 5 and 10 min. Immediately after the treatment, main procedures of comet assay were as follows: cell lysis, DNA denaturation (at pH > 13), electrophoresis at pH > 13, washing, staining DNA with SYBR GREEN I, fluorescence microscopy analysis, and processing of DNA comet images. To quantify the level of DNA damage, the parameter TDNA% (the percentage of DNA in the comet tail) was used.

Results

The level of DNA damage induced by exposure to O₃ at a concentration of 900 µg/L in the ozone-oxygen mixture for 10 min was 12.4 ± 0.8%, which is comparable to the level of DNA damage induced by exposure to gamma radiation of ⁶⁰Co at a dose of 3 Gy (12.1 ± 0.8%) (Table).

Table. The average level of DNA damage in peripheral blood leukocytes induced by exposure to ozone at concentrations of 200, 400, and 900 µg/L for 10 min and gamma-radiation of ⁶⁰Co at a dose of 3 Gy.

Ozone concentration (µg/L)			Gamma radiation
200	400	900	
2.6 ± 0.5	3.1 ± 0.8	12.4 ± 0.8	12.1 ± 0.8

Conclusion

Thus, it was found that the ozone concentration of 900 µg/L is optimal for application as a genotoxic load in the analysis of DNA damage. Using ozone instead of traditional gamma radiation, we solved a problem of development of a new test load for the comet assay.

Key words: ozone, comet assay, DNA damage

DNA DAMAGE IN RAT BLOOD LEUKOCYTES AFTER OZONE-PHOTODYNAMIC THERAPY OF EXPERIMENTAL MALIGNANT NEOPLASMS

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Introduction

For the wide use of photodynamic therapy (PDT) in the clinic, it is necessary to search for new approaches to improve its anti-tumor efficacy. Based on the knowledge of the mechanisms of PDT and the effects of medical ozone, we suggested a hypothesis about the possibility of potentiating the efficacy of photodynamic effects by ozone technologies. With such a combination, genotoxicity control is of great importance, which we propose to assess by the level of DNA damage and repair in whole blood leukocytes. The proposed hypothesis was tested in experimental models of neoplasia in rats. The genotoxic effect of anti-tumor ozone-photodynamic therapy was assessed using the alkaline comet assay.

Materials and Methods

Xenograft cancer models on 58 non-linear non-native male rats (250±25 g in body weight) were used. Transplanted sarcoma RA was used as a neoplasia model. Rats were divided into four groups: "Intact" – healthy animals (n=14), "Control" – animals with subcutaneously transplanted sarcoma RA without treatment (n=14), "PDT" – rats with neoplasia treated with PDT (n=15), "PDT + ozone" – rats with neoplasia treated with both PDT and injections of ozonated physiological saline (OPS) with ozone concentration of 400 µg /L in the ozone-oxygen mixture (n=15). The tumor volume varied from 0.5 to 4.2 cm³ in experimental animals. The photosensitizer "Photosens" (NIOPIK, Russia) was introduced intratumorally. The anti-tumor effect of the therapy was evaluated using the coefficient of the absolute tumor growth. DNA damage and repair in blood leukocytes was assessed using the authors' modification of comet assay (Chernigina and Shcherbatyuk, 2016). To quantify the level of DNA damage, the percentage of DNA in the comet tail was used (Sirota and Kuznetsova, 2010).

Results and Discussion

Analysis of data on the intensity of transplanted tumor growth showed statistically significant inhibition of neoplasia growth only at combined use of PDT with OPS injections (table). At the same time, the average level of spontaneous DNA damage of blood leukocytes in experimental groups was practically the same. However, the analysis of reparative capabilities of blood leukocytes after treatment by ozone test load revealed an increased average level of residual DNA damage in "PDT" group, indicating a violation in the

DNA repair system and probably genotoxicity. At the same time, no such phenomenon was observed after the combined action of PDT with OPS injections, which indicates to low genotoxicity.

Table. Changes of different indices in animals after treatments

<i>Groups</i>	<i>Coefficient of the absolute tumor growth</i>	<i>Spontaneous DNA damage, %</i>	<i>Residual DNA damage after test load, %</i>
<i>Intact</i>		4.3 ± 0.3	11.3 ± 0.6
<i>Control</i>	7.57 [3.57; 11.63]	5.8 ± 0.6	13.7 ± 1.0
<i>PDT</i>	1.92 [-1.0; 17.26]	4.2 ± 0.7	38.5 ± 5.5*
<i>PDT + ozone</i>	0.42 [-0.21; 3.49]*	7.6 ± 1.0	9.7 ± 1.4

Note. Data for Coefficient of the absolute tumor growth are presented as Median [25%, 75%], data for DNA damage are presented as M ± SE. * - statistically significant differences compared to the "Control" group by the Mann-Whitney *u*-test ($p < 0.05$).

Conclusion

Thus, the combined ozone- photodynamic therapy has the strongest anti-tumor activity and low genotoxic effect on whole blood leukocytes of cancer rats compared with PDT alone.

Key words: photodynamic therapy, ozone therapy, DNA damage, comet assay, experimental oncology

THE STATE OF OXIDATIVE METABOLISM IN LUNG AND HEART OF RATS AFTER A PROLONGED COURSE OF NITRIC OXIDE INHALATIONS

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Introduction

Nitric oxide (NO) is a universally recognized universal regulator of cellular and tissue metabolism. Using NO in biology and medicine with sanogenetic purposes, it is necessary to remember the problem of intensification of free radical oxidation (FRO).

The **aim of the work** was the assessment of the state of pro- and antioxidant systems in the lungs and heart of rats after a long course of inhalation of different concentrations of nitric oxide.

Material and methods

The experiment was conducted on male Wistar rats. 7 rat groups were formed: 1 – control (healthy animals, n=8); 2, 3, 4 – experimental (n=5 in each), rats were subjected to inhalations of 20ppm, 50ppm and 100ppm NO (10 min) for 30 days, respectively; 5, 6 and 7 – experimental (n=5 in each), animals 30 days were daily inhaled for 10 minutes by 20ppm, 50ppm and 100ppm NO and then 30 days were not subjected to manipulation. Rats of 2, 3 and 4 groups were removed from the experiment on the 30th day, rats of 5, 6, 7 groups-on the 60th day under anesthesia. Synthesis of the gas mixture was produced using an experimental apparatus for generating NO (Sarov). FRO and total antioxidant activity (TAA) were studied at BHL-06 (N. Novgorod), malonic dialdehyde (MDA), superoxide dismutase (SOD) and catalase activity were determined in cytosolic and mitochondrial fractions of the lungs and heart. The results were processed using Statistica 6.0.

Results and discussion

There was an increase of FRO, MDA and TAA in the cytosol and mitochondria of the lungs after use of NO (days 30). On days 60 the FRO significantly decreased in mitochondria and cytosol of the lungs after the application of NO in the concentration of 50ppm and 100ppm. After the recovery period, the MDA content decreased in the cytosol and mitochondria of the lungs at all investigated concentrations of NO. The use of NO for days 30 resulted in an increase in TAA in the mitochondria of the lungs with all NO concentrations. On the 60th day of the TAA remained elevated in the cytosol of the lungs. NO after days 30 of inhalation increased the activity of SOD and catalase in lung rats especially at 20 ppm NO.

On the 30th day of the experiment, an increase in FRO and MDA was observed in the cytosol of the heart using all NO concentrations, most pronounced at 100ppm. There was a tendency to normalization of FRO and MDA concentration in the heart mitochondria of rats of groups 5, 6 and 7. NO (20ppm) after a 30th day course of NO inhalations had a stimulating effect on TAA in the cytosol of the heart, which increased after 60 days. In this case, the stimulating effect of all NO concentrations on TAA in the mitochondria of rats of 2-7 groups was revealed. Inhibition of SOD and catalase activity under the influence of 50 and 100ppm NO in cytosol and mitochondria of rat heart in 30 days after inhalation was noted.

Conclusion

Thus the prooxidant effect of NO was revealed in mitochondria and cytosol of the lungs and heart of rats after days 30 inhalation-external application of NO. There was the increase in the activity of SOD and catalase in the mitochondria of the heart of rats in the recovery period (60 days).

Key words: nitric oxide, free radical oxidation, antioxidants

OZONE THERAPY IN SMALL ANIMALS VETERINARY MEDICINE.

PRACTICAL APPLICATION

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The highlighting of the connections between oxidative therapy protocols, practical target pathology and clinical results, under the specific limited conditions of small animal veterinary medicine in a private business, was performed.

Ozone generators, oxygen/ozone quality control modes, ozone therapy protocols increased effectiveness by adding light blood irradiation and practical results are exposed in full pictures.

Controversial issues about ozone therapies regarding D.I.V. and i.v. U.V. laser treatment are described.

These findings have significant implications for undervalued protocols in human medicine (ex: bag treatments in military and civil traumatology medicine) and industrial growth of large animals (ex: D.I.V. is a fast, cheap and efficient protocol for infectious diseases).

The results obtained by applying oxidative therapies, with or without phototherapy, offer astonishing medical solutions, some of them with very low financial impact.

Key words: veterinary ozone therapy, direct intravenous ozone, ultraviolet laser therapy intravenous, ozone bag treatment

OZONE THERAPY IN A COMPLEX TREATMENT OF PATIENTS WITH ATHEROSCLEROSIS

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The aim of our research has been to study efficacy of ozonotherapy integrated in the complex spa treatment of patients suffering from atherosclerotic lesion of vessels located in certain organs, and to suggest appropriate schemes and methods of ozonotherapy at the given nosology, in the given spa treatment programs.

Our examination has covered 185 patients with atherosclerotic lesion of arteries in certain parts of organism and 355 cardio patients with arterial hypertension (AH) and ischemic heart disease (IHD).

Methods of research: clinical and biochemical blood tests (to define the parameters of lipid and carbohydrate metabolism, as well as of coagulation system).

The course of ozonotherapy consisted of intra-vein capillary infusion of ozonated saline (OS). Patients in the control groups received complex spa treatment.

While analyzing lipidogram, we have witnessed a clear fall by 18,4% of the level of cholesterol, as atherogenic fractions of lipoprotein of low density and of very low density have been reduced by 28,7%, amount of triglycerols has decreased by 17,2%, and index of atherogeneity has dropped by 24,1%.

Table 1. Data on the clinical efficacy of ozone treatment for atherosclerosis.

Location of the process	Efficacy of treatment		
	Improved	Satisfact.	Unsatisfact.
Atherosclerotic lesion of vessels	71 pers. 69,6%	28pers. 27,5%	3 pers. 2,9%
Atherosclerotic lesion of vessels of the lower extremities	65 pers. 78,3%	14 pers. 16,9%	4 pers. 4,8%
Atherosclerotic lesion of vessels in cardiac patients (IHD) and (AH)	Ischemic heart disease		
	185pers. 84,5%	28pers. 12,8%	6pers. 2,7%
	Arterial hypertension		
	122pers. 89,7%	11pers. 8,8%	3pers. 2,2%

Analysis of the changing parameters of coagulograms has revealed the hypercoagulation effect caused by low concentrations of ozone applied in patients' treatment. In cases of ozonotherapy there was noted a clear decrease in the amount of fibrinogene by 17,0 %. At the same time there was a clear decline in the

parameters of free and induced aggregation of thrombocytes along with the noticeably increasing fibrinological activity of plasma.

Clinical efficacy of ozone therapy in rehabilitation of cardio patients and patients with atherosclerotic lesion of vessels in different organs at the stage of spa treatment has been assessed as high (table 1), which implies further perspectives of integration of this method in rehabilitation programs provided to such patients.

Keywords: atherosclerosis, ischemic heart disease, ozone therapy

SOME RESULTS OF THE OUTCOME AFTER OZONE THERAPY DIAGNOSED WITH DIABETES

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Introduction

Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the heart and blood vessels. It became clear that ameliorating oxidative stress through treatment with antioxidants might be an effective strategy for reducing diabetic complications. Medical ozone treatment useful in the treatment of diabetes and its complications.

Materials and methods

The study was designed as selective sampling and clinical trial study. There were enrolled 30 patients with diagnosis of diabetes type 2 at Wellness Med Hospital in Mongolia. Patients were used by Ozonatte medical ozone generator for major autohemotherapy (MAH). In this course, there were almost 10-12 treatments. The low intensity millimeter wave (MW) generator, and acupuncture for ear points were the supporting treatment for complications. All complications, HbA_{1c} were checked before and after course of treatment. Also every treatment day glucometer test was done.

Results

47 percent was male, 53 percent was female and the mean age was 55.13±10.62. After the course therapy 100 percent of them stopped complaints about being thirsty, chronic fatigue, insomnia and 30-70 percent of them reduced the following complications frequent urination, skin itching, dry in a mouth tingling sensation. And by the time the course ended, the glucose level lowered to almost 30 percent.

Conclusion

This study shows that HbA_{1c} (p=0.014) were decreased with statistically significant by therapy of ozone may have an additive effect in the treatment of diabetes; this may attribute to the multiprotective antioxidant effect of ozone.

MW and acupuncture therapy well combined with ozone therapy for decreasing complications.

Keywords: antioxidant, diabetes, ozone, major autohemotherapy, low intensity millimeter wave, glycated haemoglobin HbA_{1c}

EVALUATION OF INTEGRAL INDICATORS OF SYSTEMIC INFLAMMATORY REACTION IN PATIENTS WITH SURGICAL INFECTION OF SOFT TISSUES WHEN USING OZONE THERAPY

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The study assesses the effectiveness of ozone therapy in the complex treatment of surgical infection of soft tissues using the main indicators of the wound process (terms of cleansing wounds from fibrin, the appearance of granulations, marginal epithelization, the average daily percentage of area reduction), microbial contamination of wounds, criteria for systemic inflammatory response syndrome. The study included 545 patients: 225 patients in the study, where in combination with traditional treatment was carried out ozone therapy and 290 in the control group who received only traditional treatment. On the first, fourth, tenth days the study of indicators was carried out.

We observed a statistically significant improvement in wound healing process, microbial contamination, and a tendency to normalization of the main criteria of systemic inflammatory response syndrome on the background of the use of ozone therapy in comparison with traditional treatment.

Key words: ozone therapy, wound process, systemic inflammation response syndrome, phlegmons of extremities

THE USE OF OZONE THERAPY IN CHRONIC OBLITERATING DISEASES OF LOWER LIMB ARTERIES

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The paper shows the results of complex therapy of 154 patients with III degree of ischemia according to Pokrovsky A.V. classification. Patients underwent ozone therapy by introducing 200 ml of a physiological solution with ozone concentration of 0.7 mg/l in the solution. For ozone production the ozonator "Medozons BM" was used. The course of treatment was 10 procedures. The courses were held 2 times a year.

Results of treatment are traced in terms of 1 year to 5 years. Courses of treatment were carried out 2 times a year. In the study of microcirculatory parameters at admission and on the 10 day says significant improvement under the influence of the studied indicators: the index of effectiveness of microcirculate, increased microcirculation in comparison with the values at admission. In 70% of patients, there was a regression of symptoms (chills and cold feet of the lower extremities, a decrease in seizures). In the study of walking distance, 40% of patients had an increase in it.

Key words: ischemia, ozone therapy, chronic obliterating disease