Ozone Correction Of Metabolism Misbalance Induced By Endogen Intoxication In Patients With Burning Injury

C. Kontorschikova[1], S. Peretyagin[2], I. Okrut, A. Bavrina, J. Efremenko, O. Kostina

1. The State medical Academy of Nizhny Novgorod, Nizhny Novgorod, Russia
2. Medical Center for Burnt Patients Nizhny Novgorod, Russia

Key-words: ozonetherapy, burnt patients, lipid peroxidation, modified proteins, nitric oxide, proteolytic activity.

Abstract

The development of new methods as well as the improvement of the current management of burnt patients is of great importance in order to low down the lethality level. Ozonetherapy was found to be one of these methods and proved to be efficient in various diseases. There were revealed ozone mechanisms that are effective in the management of burnt patients such as bactericidic, antihypoxic, analgetic, antiflammatory, immunomodulating, detoxicating. They also include hemostatic correction and normalisation of pro- and antioxidant systems ratio.

The aim of the study was to assess the effect of ozone in a complex treatment of patients with burning injuries

There were examined 41 patients, 25 patients were on conventional therapy and made up the control group. The clinical trial group of 16 patients in addition to complex therapy received 10 procedures of autohemotherapy with ozone dose of 250μg.

Ozone efficiency was assessed by using the following criteria:1) general condition of a patient, 2) the rate of microbic vegetation in burning injuries, 3) findings of clinical and laboratory examinations and primarily, analysis of lipid peroxidation indices, the level of modified proteins due to oxydation and the level of nitric oxide by the content of its final products(nitrites).

Clinical observations showed significant ozone effect in the management of burnt patients that were manifested by a quicker diminishment of edema and perifocal inflammation and by earleir epithalization. Secvestration of necrotic area was noted much earlier in cases with deep burning wounds, the terms for necroectomia were shorter and autodermoplastics could be performed sooner.

The rate of microbic dissemination of the burnt area was vividly diminished.

All burnt patient initially had decreased levels of serum albumin, while the levels of globulins, blood urea and of alanine aminotransferase were increased, the latter had a 2-fold increase. The patients that were on ozone treatment showed significantly more pronounced normalization of the aseessed blood parameters.

Thermal injuries cause a number of biochemical reactions producing active forms of oxygen and of nitric oxide. It results in the increase of chemiluminogram indices(Imax, S, I/S, tg2) in patients' serum. Molecular products of lipid peroxidation, both primary – dien conjugates(DC), trien conjugates (TC) and the final ones- Shiff bases(SB)are also found to be increased as well as modified proteins. Of a particular interest was the double increase of nitrites' level- final products of nitric oxide. High concentrations of nitric oxide do not produce their action through (c-GMP) as they do it in normal condition. On the contrary, they produce direct cytotoxic and immunogenic effect, binding with superoxide and forming peroxinitrite, that induces DNA disorder, inhibits enzymes function. In this case nitric oxide can be regarded as a factor of endogenic intoxication playing an importanr role in the course and the outcome of critical conditions.

The clinical trials showed the burnt patients to have LP activity correlative with the nitrites content in serum, the content of proteins peroxidation correlating with the burnt area.

The increased activity of proteolytic serum enzymes-trypsin (2.6-fold), elastase (10-folds) with 4-fold decrease of inhibitor alpha-1 –antitrypsin seems to be important [11].

The use of ozonetherapy in the trial group was found to normalize the lipid peroxidation indices in a much shorter period of time and to decrease the quantity of modified proteins and nitrites. Simultaneously, proteolytic enzymes and their inhibitors were coming back to the initial balance, while in the control group proteolytic activity remained 1,5-fold above the normal level.

The observed changes can be regarded to produce a positive effect in the correction of metabolic disturbances resulted from burnt endotoximia and prove ozone to be efficient for patients with burning injuries.

Introduction

Development of new methods together with the improvement of the existing ones is of great importance for the treatment of burn patients and decreasing the lethal outcome. Ozonetherapy can be one of these methods, for it has already proved its efficiency in many diseases and has provided scientific basis for the mechanisms of ozone effect such as bactericidal, anti-hypoxic [6,9,11], analgetic, anti-inflammatory, detoxicating, immune-modulating [15]. They also include hemostatic [13] correction and normalisation of pro- and antioxidant systems ratio [9, 10].

Medical ozone was found to play an important role in haemostasis correction and normalization of the pro- and antioxidant ratio. All these mechanisms proved to be very helpful in the management of patients with burning injuries. Neurologists and cardiologists having used Doppler-cardiography revealed an evident vasodilatation effect that occurred after parenteral infusions with ozonated physiological saline [3, 6]. Vasodilatation effect could be explained by synthesis of nitric oxide.

This work was done to evaluate the efficiency of ozonetherapy combined with a complex treatment of patients with burning injuries.

Material and methods

Clinical trial was done in the Center for Burn Patients in Nizhny Novgorod Research Institute of Traumatology and Orthopedics. There were examined 41 patients, age ranging 25-40, with burns of the II-III-a grades. The patients of the control group (25 persons) were treated with conventional methods. In addition to conventional therapy 16 patients in the control group had 10 procedures of major autohaemotherapy with ozone dose of 250 mcg.

Ozone efficiency was assessed by using the following criteria:1) general condition of a patient, 2) the rate of microbic vegetation in burning injuries, 3) findings of clinical and laboratory examinations and, primarily, analysis of lipid peroxidation indices, the level of modified proteins due to oxidation and the level of nitric oxide by the content of its final products(nitrites).

The nitric oxide in blood was calculated by the content of the final products of its metabolism – nitrites, that were found in venous blood according to P.Golikov's method (2004). The method is based on reddish coloring of nitrite solution due to Griess reaction with the reagents of sulfanilamides and N-(1-nafti)-ethylene diamine dihydrochloride(NEDA). The solution absorbtion was measured with the wavelength of 250 ηm. Sodium nitrite was used for standard [1].

Complex assessment of lipid peroxidation processes included 1) analysis of induced biochemiluminescence readings using БХЛ-06 biochemiluminometer, regarding I max and S indices, that identify potential capacity of biosubstrate for LP and characterize total antioxidant activity, respectively. [5], 2) estimation of lipid peroxidation products: primary products – dien (DC) and trine conjugates(TC) and final ones – Shiff bases(SB).

Peroxide protein modification was assessed according to Dubinina's method, which is based on reaction of oxidized protein amino-acid residue with 2,4 dinitrophenylhydrasine (2,4-DPH) producing 2,4-DPH derivatives. Protein oxidation results in aldehyde and ketone groups of amino acid residues which interact with 2,4-DPH and have absorption spectrum in the range of 260-558 nm, 258-264 nm, 458-520 nm [2].

Results and discussion

Clinical observations showed marked efficiency of ozone treatment in patients with burning injuries, that could be seen in a more rapid edema and peri-focal inflammation elimination and earlier epithelization. In extensive burns there were noted shorter periods of sequestration of necrotic area and of necroectomia, that enabled the physicians to perform autodermatoplastic surgery earlier .
The burnt surface inseminated with microorganisms was also significantly lessened.

All patients initially had lowed albumin levels, but elevated levels of globulins, blood urea and double activity of alanine amino-transferase. In patients that were on ozone treatment normalization of the tested blood parameters was more pronounced compared with the control group.

Thermal trauma is known to cause the development of a complex of biochemical reactions, generating active forms of oxygen and nitric oxide. In the pathogenesis of complications due to thermal burns of great importance is the condition of endothelium, that together with other active agents synthesizes nitric oxide. The main target for nitric oxide is known to be soluble guanilatcyclase, containing the gem that acts as a receptor for nitric oxide. On being bound this receptor induces activation of soluble guanilatcyclase, accompanied by enhancement of cyclic guanosin-monophosphate biosynthesis(c-GMP) [1, 3]. c-GMP decreases the concentration of intracellular calcium in smooth muscle cells, that causes their relaxation and vasodilatation [4]. Reaction of nitric oxide with oxygen results in producing nitrite and nitrate, stable final products that are the main markers for nitric oxide in blood [1].

CONTROL	BEFORE THE TREATMENT	AFTER THE TREATMENT
6,8±0,46	16,9±1,26	$9,3\pm0,71^{+}$

Table1. Nitrite content in blood of burn patients before and after ozone treatment (mcm/l)

- differences are valid compared with control group: $^{+}$ - $p<0,05$,

The level of nitric oxide is found to have a steep rise in pathologies caused by burns [3,6], nitric oxide synthases activated in macrophages and hepatocytes, augmenting its production as much as 1000 times [1]. High concentrations of nitric oxides do not produce their effect through c-GMP.

On the contrary, they produce direct cytotoxic and immunogenic effect, binding with superoxide and generating peroxinitrite, which induces DNA disorder and mutation, inhibiting enzymes function [10,11] High nitric oxide concentrations do not produce their effect through c-GMP compared with the normal condition. In this case nitric oxide, being a factor of endogenic intoxication, plays an important role in the course and outcome of critical conditions.

These changes were observed in activation of lipid peroxidation processes and resulted in raised levels of chemiluminogram readings of patients blood (Imax, S, I/S, tg-2), lipid peroxidation molecular products - primary ones- dien conjugates (DC), trine conjugates (TC) and final ones – Shiff bases(SB) [7,8,14] and modified proteins.

CONTENT OF OXIDATION PRODUCTS			
	356nm	363nm	370 nm
BEFORE THE TREATMENT	20,3+ 0,026	20,9+ 0,042	20,1+0,025
AFTER THE TREATMENT	11,5+ 0,032	12,1+ 0,025	12,2+0,031
CONTROL	11,5+ 0,032	5,5+0,023	5,4+0,023

Table 2. Content of neutral alymphatic aldehyde - and ketone- dinitrophenylhydrasones in the serum of burn patients

Of particular interest was the double-fold increase of nitrites – final products of nitric oxide. Patients with burning injuries were found to have a rapid intensification of LP processes, that could be explained by the fact that any inflammation is regarded as one of the most powerful factors inducing LP processes. The elevation of nitrite, the final product of nitric oxide, revealed in patients with thermal trauma coincides with the findings reported by other authors.

Our clinical trials showed that the patients with burns had LP indices correlating with nitrites content in blood. The content of protein peroxidation was relative to the burnt area. Of important significance was the increase in the activity of serum proteolithic enzymes: trypsin – 2.6 times, elastatse – 10 times with the decease of anti-trypsine – 4 times.

Groups	Imax	S	tg –2a	DC	TC	SB
Before the treatment	2,5+0,17;	26,3+3,9	-0,915 +0,02	0,36+0,01	0,102+0,01	7,9+0,5
After the treatment	1,7+0,12	19,1+1,7	-0,455+0,01	0,21+0,01*	0,091+0,01	4,6+ 0,6*
Control	1,2+0,11	15,6+1,1;-	0,365+0,01	0,18+0,02	0,050+0,01	3,9+0,42

Table 3. Lipid peroxidation indices before and after ozonetherapy in patients with burns

- differences are valid compared with control group: $^{+}$ - p<0,05,

The course of ozonetherapy in the trial group made it possible to normalize the parameters of lipid peroxidation and lessen the amount of modified proteins and nitrites in a much shorter period of time compared with the control group. We could also observe the resuming balance of proteolytic enzymes and their inhibitors, while in the control group proteolytic activity remained 1,5 times higher than the normal one

The observed changes could be regarded as positive in the correction of metabolism disorders induced by burning endotoxemia. And at the same time they can provide sufficient evidence for the efficiency of ozonetherapy for the patients with burning injuries.

Conclusion

Ozonetherapy in patients with burning injuries results in enhancement of antioxidant defense system and, hence, reduces the intensity of protein and lipid peroxidation, eliminating the nitrites quantity.

References

1. Голиков П.П. Оксид азота в клинике неотложных заболеваний // Медпрактика. - Москва. - 2004. - 75с.

2. Дубинина, Е.Е.Окислительная модификация белков сыворотки крови человека, метод ее определения / Е.Е. Дубинина [и др.] // Вопросы медицинской химии.-1995.-Т.41, №1.-С.24-26.

3. Костина О.В. Состояние про- и анти-оксидантных систем организма при ожогах в условиях воздействия озоном и биосканом С. - Автореф. дисс.к.б.н.. - Н.Новгород. - 2001.

4. Котов С.А., Конторщикова К.Н., Густов А.В. Средство нормализации системы перекисного окисления липидов мозга. Патент на изобретение № 2109512 . - Москва. - 27.04.1998г.

5. Кузьмина Е.И., Нелюбин А.С., Щенникова М.К. Применение индуцированной хемилюминесценции для оценки свободнорадикальных реакций в биологических субстратах // Межвузовский сборник биохимии и биофизики микроорганизмов. - Горький,1983. - С. 179-183.

6. Перетягин С.П. Механизмы лечебного действия озона при гипоксии // Озон в биологии и медицине: Тез. докл. I науч.-практ. конф. - Н. Новгород, 1992. - С.4-5

7. Fletcher D.L., Dillared C.J., Tappel A.Y. Measurement of fluorescent lipid peroxidation products in biological system and tissues // Analyt. Biochem. - 1973. - Vol. 52. - P. 497-499.
8. Folch J., Less M., Stanley A. A simple method for the isolation and purification of total lipids from animal tissues // Biol. Chem. - 1957. - Vol. 226. - № 2. - P. 497-509.
9. Kontorschikova C.N. Biochemical safety control in ozonetherapy // Proc. of the 12th World Congress of International Ozone Association. - Vol. 3.- Lille, France. - 1995. - P. 231-234.
10. Kontorschikova C.N. Ozone in medicine. Proceedings of the 11-th Ozone World Congress. - San-Francisko. - 1993. - P. 3-45.
11. Kontorshchikova C. N., Yefremenko J.R. BIOCHEMICAL PROPERTIES OF OZONETHERAPY // Proceedings of 4th International symposium on Ozone Aplications - Cuba - april 2004.
12. Kuzmina E.I., Kontorshikova C.N., Maslennikov O.V. Peroxide Resistence of Eritrocyte Membrane during Ozonotherapy // 13th Ozone World Congress. - Japan, Kyoto. - 1997. - P. 243-244.
13. Okrut I., Kontorshchicova C. Dose-dependent ozone effect on thrombocytic and coagulation hemostasis in vitro. // Ozone in medicine. Proceedings of the 13th Ozone World Congress. - Osaka. - 1997. - P. 130-131.
14. Shenstone F.S. Spectrometric identification of organic compounds. Ultraviolet and visible spectroscopy of lipids. - 1971. - ¹ 5. - P. 77-91.
15. Studies on the biological effects of ozone: 3. An attempt to define conditions for optimal induction of cytokines/ Bocci V., Luzzi E., Corradeschi F., Paulesi at al.//Lymphokine Cytokine Res.-1993.-№12.-P.121-126.