Ozonetherapy in a postoperative period of oncological patients

E. Kontorshchikova[1], T.Kachalina[1], G.Grechkanev[1], V.Novikov[2]

[1]The State Medical Academy, Nizhny Novgorod, Russia
[2]Nizhny Novgorod State University named after N. Lobachevsky, Nizhny Novgorod, Russia

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Abstract

The incidence of onco-gynecological diseases is one of the highest among female scourges involving different age groups. The primary importance at the early stages of treatment is conventionally given to surgical intervention followed by polychemio-therapy, hormonal and radial therapies. In these conditions the use of new methods that might correct the levels of antioxidant defense system and of lipid peroxidation as well as to modulate immunological response seems to be very promising. The aim of this study was to evaluate ozone effect on various homeostatic parameters in postoperative period.

There have been examined 100 women with various onco-gynecological diseases (cervical carcinoma, ovarian carcinoma, endometrial carcinoma). All the patients were compatible according to the age and concomitant pathology and were operated for the pathology. Ozonetherapy course of 6 procedures,(ozone concentration of 400 µg/l in physiological saline) done intravenously every second day was administered to 60 women, starting on the second postoperative day. Patients of the control group (40 patients) were on conventional management without ozone administration
In addition to clinical examination, all the patients were taken tests for intensity of serum lipid peroxidation according to the indices of chemilumenescence - primary (dien conjugates), secondary (trien conjugates) and final (Shiff bases) products and for general antioxidant activity.

To evaluate the immune status the mononuclear cells of peripheral blood were immunophenotyped regarding the amount of CD 4, CD8, CD16, CD22, CD25, CD71, CD 38, CD95, HLA 1, HLA DR. The levels of soluble antigens : CD 50, CD95, CD38, CD54 as well as the antigens of the histocompatible classes (HLA -1 and HLA - 2) were also analyzed. Competitive solid-phase enzyme immunoassay was used to identify the levels of tumor necrosis factor(TNF) and of interleukin-2 (Il –2).
Ozonetherapeutic procedures were not found to cause any complications. The clinical effect could be seen in improvement of appetite, better sleep, alleviation of nausea and weakness. All patients with elevated bilirubin, fibrinogen and hepatic enzyme activity on having undergone the course of ozonetherapy were found to have these indices returning to baseline value.
Initial laboratory results showed the increase in total antioxidant blood activity and the depression of lipid peroxidation processes. Taking into consideration ozone property at low concentration to improve the immunity and lipid peroxidation, ozone was used as a poliative remedy.
Ozonetherapy proved to be very helpful in the management of chemiotherapy complications in patients with onco-gynecological pathology.

Introduction

The incidence of onco-gynecological diseases is one of the highest among female scourges involving different age groups the world over and tends to be increasing. An important part in the pathogenesis of these conditions belongs to misbalance between pro- and antioxidant systems and changes in the immune components ratio. In the management of these patients at the early stages the primary attention is conventionally given to surgical intervention followed by cytostatic or and radial therapy.

Chemio- and radiotherapy is known to cause intensification of oxidative processes with depression of compensatory functions of antioxidant defense system (ADS) with the development of immune insufficiency. In these conditions the use of new methods that might correct the levels of antioxidant defense system and of lipid peroxidation, to modulate immunological response, to produce desintoxicating effect and have no side effects - seems to be very promising. Ozonated physiological saline(OPS) was found to meet all these requirements.

The aim of the present study was to provide a pathogenetic basis for the use of medical ozone in women with onco-gynecological diseases during the post-operative period. Ozonetherapy was regarded as an auxiliary course to prepare the patients for the second stage of combined treatment with cytostatic and radiotherapy.

Material and methods

The clinical trial was performed on 100 patients with onco-gynecological pathology of various localization and surgical intervention done at the first stage of the combined management.

The first group consisted of 60 patients who during post-operative treatment in addition to the conventional treatment with analgesics, antibiotics and hypocoagulants, were administered intravenous instillated infusions of ozonated physiological saline (ozone concentration – 400mcg/l). The course of ozonetherapy included 6 procedures of 200ml of OPS, done every second day, starting from the second post-operative day. The ozone concentration, the number of the procedures and the saline volume had been tested and proved to be efficient and safe by the previous studies done and reported by gynecologists, oncologists and therapists [1-4]

The second group (the control one) was made up of 40 patients that were on conventional post-operative therapy without ozone. Regarding the ozone properties to normalize the balance of lipid peroxidation products and antioxidant defense system, as well as its immune-modulating effect, we chose these homeostatic parameters as being pathogenically appropriate for the trial with onco-gynecological patients.

Free-radical oxidation in the patients' blood was evaluated by the following chemilumenogramme parameters: I max - potential patient's capacity for lipid peroxidation; S – the 30 seconds chemiluminescence's sum light, the anti-oxidant defense retroactive value; tg(-2α) – an anti-oxidant system index, showing the decreasing rate of free radicals oxidation in serum. To make a more profound investigation of LP processes we estimated the content of lipid peroxidation molecular products: primary – dien conjugates(DC), trien conjugates (TC) and final ones –Shiff bases(SB)

Parallel to those we studied the parameters of cellular and humoral immunity with the help of the latest methods of lymphocytes morphological phenotype, using the panel of monoclonal antibodies and analysis of the most important cytokine levels- TNFα(tumor necrosis factor) and interleukin-2, that are known to link the components of the immune system.

Results and discussion

Lately there have appeared a great number of reports on pro- and antioxidant misbalance to play an important role in the genesis and course of various diseases, including the oncological ones

[5,6]. Due to oxidative stress LP toxic products on getting accumulated in the body cause misbalance of homeostasis regulation, serious metabolism disorders, changes in the metabolic status and various shifts in the functions of different systems. It was found that the intensity of free radical reactions at the early stages of malignization increases, while during the later stages they get suppressed. The similar phenomenon was observed in our work.

The initial state of the pro- and antioxidant indices in onco-gynecological patients was misbalanced and was to be corrected. In both groups. S index exceeded the norm ($p \leq 0,05$), being 18,42±0,738 mv in the control and 19,37±0,810 mv – in the main one. After the course of ozonetherapy this index lowed ($p < 0,05$) to the baseline level from 19,37±0,810 mv to16,49±0,724 mv, a tg (-2α) was also decreasing($p < 0,05$) from -0,346±-0,006 to 0,257±-0,0049. Activation of antioxidant defense system resulted in efficient decrease of I max from 1,79±0,101 mv to 1,33±0,100 mv and as a consequence - the reduction ($p \leq 0,05$) of lipid peroxidation primary products - TC(from 0,056±0,0085 to 0,030±0,0071).
There were no changes in DC content (0,157±0,024); insignificant diminution was found in SB (from 4,98±0,59 to 4,82±0,62)
In control group patients after the operation and conventional post-operative management I max was increasing($p \leq 0,05$) from1,54±0,106 mv to 2,13±0,110 mv. S index had an increasing tendency(from18,42±0,738 to19,56±0,815 mv), tg(-2α) was elevated ($p \leq 0,05$)compared with pre-operation condition(from –0,324±-0,007 to –0,511±-0,0069).

CL indices \ Groups of patients		Control group N=40	Main group N=60	Norm
I max (mv)	Before the treatment	1,54± 0,106	1,79±* 0,101	1,0 – 1,5
	After the treatment	2,13±** 0,110	1,33±** 0,100	
S (mv)	Before the treatment	18,42±* 0,738	19,37±* 0,810	12 - 15
	After the treatment	19,56± 0,815	16,49± 0,724	
tg(-2α)	Before the treatment	-0,324± -0,007	-0,346± -0,006	-0,28 – -0,5
	After the treatment	-0,511±** -0,0069	-0,257±** -0,0049	

Table 1. Chemiluminogramme changes before and after the treatment

*Note: * - Difference efficiency compared with the norm ($p \leq 0,05$),*

*** - Difference efficiency compared with initial state ($p \leq 0,05$).*

With simultaneous elevation of Imax it became evident that antioxidant defense kept decreasing as a consequence of operation and the lack of special antioxidant support.
At the same time there could be seen the tendency to elevation in the LP product levels –DC and TC, and augmentation of SB ($p \leq 0,05$) compared with the initially elevated level ((4,93±0,41) that could be regarded as unfavorable prognostic sign for these patients.
Thus, the use of ozonetherapy in a combined management of post-operative onco-gynecological patients was shown to eliminate the misbalance between pro- and antioxidant systems that is displayed by the decrease of lipid peroxidation intensity and increase of antioxidant serum activity.
The received result can be regarded as a pre-condition for reinforcement of immunity and better tolerance to polychemio- and radial therapy.

LP indices / Groups of patients		Control group N=40	Main group N=60	Norm
Dien Conjugates	Before the treatment	0,183±,027	0,157±,026	0,15-0,18
	After the treatment	0,218±∗0,031	0,157±0,024	
Trien Conjugates	Before the treatment	0,061±∗0,0094	0,046±∗0,0085	0,025-0,035
	After the treatment	0,064±∗0,0089	0,030±∗∗0,0071	
Shiff Bases	Before the treatment	3,55±0,61	4,98±∗0,59	2,5-4
	After the treatment	4,33±∗∗0,50	4,82±0,62	

Table 2.The content of lipid peroxidation products before and after the treatment

Note: ∗ - Difference efficiency compared with the norm ($p \leq 0,05$),

∗∗ - Difference efficiency compared with initial state ($p \leq 0,05$).

On evaluation the immune status of onco-gynecological patients at the early stages we could observe the following data: CD4+ level going down with CD8+ going up and elevation in the level of the tumor necrosis factor (TNF) T-lymphocytes-helpers or CD4+ - they are the cells, responsible for regulation of the immune response to a foreign antigen; for the control of antigen homeostasis and for augmentation of antibodies production. Diminution in T-lymphocytes-helpers is a sign of immune insufficiency.

CD8+ -T-lymphocytes-suppressors, inhibiting the immune response. T-suppressors inhibit the production of antibodies due to the delay in B-lymphocytes proliferation and differentiation and due to the development of slow hyperaesthesia. Increase in CD8+ signifies the immune insufficiency.
The leading role in evaluation of immune system belongs to helpers/suppressors ratio in peripheral blood –the so called RIA- index (ratio immune assay- CD4+ / CD8+) This index was found to be - 1,7 in the main group and 1,9 in the control one. Natural killers (CD16+) -effector- cells are responsible for antiviral, anti-tumoral and transplantation immunity. In both groups the

CD16+content was above the normal level – with 75% raise in the main group (25% being within the normal limits) and 70% - in the control one(30% staying within the norm).
We assume, that in CD4+ deficiency, the augmentation in CD16+ cells performs a compensatory function and is important for anti-tumoral protection. These cells are known as FAS-inductors in indirect apoptosis

An important role in anti-tumoral immunity is played by B-lymphocytes. In contrast to T-lymphocytes that can be activated only at early stages of tumor development, B-lymphocytes that can keep on being activated all the time. In our work B-lymphocytes are represented by subpopulations of CD22+ and HLA-DR(HLAII) cells. CD22+ level was elevated in both groups (main group -22,3±2,5%; control group -23,5±2,9%, but this increase was not efficient.
At the same time, the patients of the main (75%) and control(70%) groups were found to have an increase of HLA-DR+cells. This antigen is expressed on B-cells for all stages of differentiation, as well as on monocytes and activated T-lymphocytes. Due to the fact, that B-cells and partially T-lymphocytes participate in antigen presentation, the increase in HLA-DR+ cells level reflect active immune response to foreign antigen.

Groups of patients / Indices		Control group N=40	Main group N=60	Norm
CD3	Before the treatment	55,4±2,9	50,4±2,2	40 – 80
	After the treatment	51,7±2,6	53,5±2,9	
CD4	Before the treatment	45,5±33,8	43,5±3,1	40 – 50
	After the treatment	44,2±3,7	43,6±3,4	
CD8	Before the treatment	22,1±2,4	24,6±2,3	15 – 20
	After the treatment	19,6±2,1	22,8±1,9	
CD16	Before the treatment	28,2±1,9*	27,1±1,6*	10 – 20
	After the treatment	24,3±2,2	20,2±1,5**	
CD22	Before the treatment	23,5±2,9	22,3±2,5	10 – 20
	After the treatment	27,5±2,6	27,8±2,8	

Table 3. Cellular immunity indices before and after the treatment

*Note: * - Difference efficiency compared with the norm ($p \leq 0,05$),*

*** - Difference efficiency compared with initial state ($p \leq 0,05$).*

In evaluation of cytokines level, and precisely the tumor necrosis factor and interleukin-2 we observed the increase in these indices before the treatment: TNF was initially elevated($p \leq 0,001$) in both groups (main group – 188,6±16,6 ; control group -244,1±15,4). The levels of interleukin-2 also exceeded the norm in both groups (main group -792,7±31,2 ($p \leq 0,001$) ; control group -392,5±19,1 ($p \leq 0,001$). The average CIC level though staying within the normal range, exceeded the norm in some patients(35%) of the main and the control(33%) group.

Cytokines \ Groups of patients		Control group N=40	Main group N=60	Norm
Tumor necrotic factor	Before the treatment	244,1±15,4*	188,6±16,6*	0 – 50
	After the treatment	301,9±11,8** *	137,2±10,5*** *	
Interleukin-2	Before the treatment	392,5±19,1*	792,7±31,2*	0 – 50
	After the treatment	407,8±24,2*	439,2±29,8*** *	

Table 4. Cytokine levels in onco-gynecological patients before and after the treatment

*Note: * - Difference efficiency compared with the norm ($p \leq 0,001$),*

***Difference efficiency compared with initial state ($p \leq 0,01$),*

**** - Difference efficiency compared with initial state ($p \leq 0,05$),*

Thus, the received findings provide convincing evidence that the immune system of onco-gynecological patients in spite of the depression in some of its components still can be activated. Bearing in mind, that chemio-radial therapy produces a depressive effect on immune response, we assumed that immune-modulating therapy done in the post-operative period would improve both, the tolerance to the specific treatment and the quality of patients' life.
Immune-modulating effect, produced by ozonetherapy has been described in the works of the researchers from different countries[7,8]. That was the reason we decided to choose it in a form of intravenous infusions of ozonated physiological saline.

After the treatment we received the following results: the content of CD4+ cells in the patients of the main group remained nearly the same(before -43,5±3,1%;after - 43,6±3,4%),while in the control group it had a 3% decrease.

At the same time the levels of CD8+cells lowed down in both groups (main- 8%; control- 12%), that caused changes in RIA indices bringing them up to 1,9 in the main group and 2,2 in the control one. The increase of this index in the control group can be regarded as an unfavorable factor.

The content of CD16+ cells in the patients of the main group was decreasing ($p \leq 0,05$) and approaching the normal range, while in the control group it was insignificant and made up only 12%.

Indices \ Groups of patients		Control group N=40	Main group N=60	Norm
CD95	Before the treatment	1470±191*	856,4±61,2*	374±23
	After the treatment	1239±184*	401,8±45,3**	
CD50	Before the treatment	383,8±85,1	432,8±75,3	355±65
	After the treatment	494±50,5	278,3±69,7	
CD38	Before the treatment	550,1±91,8*	410,2±43,4*	200±17
	After the treatment	300,2±79,4	253,5±29,4**	
CD54	Before the treatment	143,3±25,5	60,1±9,1	65±10
	After the treatment	126,1±26,7	58,3±10,4	
HLAI	Before the treatment	3888±251,2*	1486±95,3*	1012±56
	After the treatment	3014±280,1	1088,1±57,4* *	
HLAII	Before the treatment	278,5±71,4*	175,7±30,3*	99±11,5
	After the treatment	201,9±75,6	105,8±28,5**	

Table 5 .Soluble factors in the peripheral blood of onco-gynecological patients before and after the treatment (U/ml).

*Note: * - Difference efficiency compared with the norm ((p≤0,01),*

*** - Difference efficiency compared with initial state (p≤0,05).*

Among the indices of humoral immunity of particular interest was 1,5 reduction(p≤0,05) of circulating immune complexes in patients of the main group, while in the control group it was increasing(p≤0,05). It can be explained by phagocytosis activation caused by ozonetherapy resulting in elimination of CIC excess. The levels of cytokines, inflammatory co-factors, were also decreasing in the main group. The TNF level in the main group had a 28% fall (p≤0,05), while in the control group it had a 19%rise (p≤0,01)

Similar changes could be seen in interleukin-2 level. In patients of the both groups this index was initially above the norm (p≤0,001) After the treatment the patients of the main group had 19% decrease(p≤0,05), while in the control group this index tended to increase (4%).The revealed phenomenon could be explained by anti-inflammatory effect produced by ozonetherapy

In patients of the main group we could observe a 36% reduction of the soluble antigen - CD50(before - 432,8±75,3 U/ml and after 278,3±69,7 U/ml) In the control group there was a tendency to augment the expression of this protein
The quantity of CD95 antigen had a 54% decrease($p \leq 0,05$) approaching the norm, while in the control group we could observe only a16% reduction.

Conclusion

Regarding the received data, OPS was producing a stimulating effect on the parameters, both of cellular and humoral immunity. OPS immune-modulating effect seems to be based on the metabolism improvement in immune-competent cells, which reinforces their tolerance to the toxicity caused by cytostatic or radial therapy.

The received data also confirmed our assumption, that medical ozone might produce a positive effect on homeostatic parameters of onco-gynecological patients during the post-operative period.

Hence, the clinical trials can provide a sufficient pathogenic basis for the use of ozonetherapy during the post-operative period in patients with malignant genital tumors can be recommended for a wide clinical practice in the health service hospitals.

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