Pathogenetical justification of ozonetherapy usage in gynecological patients after surgery

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Introduction

Malignant and nonmalignant tumors of uterine are the most common pathology in women of different age. The complex therapy of these patients includes radical surgery with hormonotherapy, polychemotherapy and radiation therapy. These methods have a whole number of side effects on a patient organism such as toxic and immunosuppressive effects as well as balance of pro- and antioxidant systems disorders [3; 5]. In this connection it is important to include in complex postoperative therapy auxiliary methods of treatment, which reduce intoxication, modify antioxidant protection level, normalize lipid peroxidation intensity and immunity. One of such methods is ozonetherapy. Treatment of a whole number of deseases including cancers demonstrates positive effects [1; 4; 5; 6; 7].

Immune system realizes elimination of tumor cells and restore the homeostasis of the organism. Reasonability of immunocorrecting therapy is identified if it changes immune cell repertoire and serum level of soluble immune cell membrane antigens or not [7; 10]. Also it is interesting to investigate lipid peroxidation process and antioxidant protection state. Some physicochemical forces can increase reactive oxygen species production and destroy tumor cells. So these forces can be used in anticancer therapy.

In this connection the aim of our research was to study the immunologic status before and after treatment with ozonized physiological saline in gynecologic patients with malignant and nonmalignant tumors for pathogenetic justification of ozone usage in persons suffering from hysteromyoma and endometrial cancer in the postsurgical period.

Materials and methods

The study population consisted of 100 gynecological patients treated in the Gynecological Clinic of the Regional Clinical Hospital of Nizhny Novgorod. Out of the 100 women, 43 had uterine myoma and 58 had endometrial cancer (adenocarcinoma of endometrium). According to the FIGO criteria all cases of cancer were stage I. the diagnosis of uterine myoma and endometrial cancer were confirmed by the examination of biopsy and cytologic specimens. The prevailing concomitant diseases were hypertension (70% ofpatients), coronary heart disease (30%), obesity (20%) and insular diabetes (15%). All patients were submitted to the radical surgery including supravaginal uteroctomy. The study population was subdivided into two groups. The control group of patients, including 22 patients with uterine myoma and 26 endometrial cancer patients (the median age was 58.7 years, range 28 - 79), received a standard postsurgical therapy for the treatment. The treatment of the main group of patients, 20 with hysteromyoma and 32 with endometrium cancer (the median age 60.3 years, range 28 - 79), combined the standard therapy and intravenous infusion of ozonized physiologic saline (0,9% NaCl). Physiologic saline were bubbled with gas mixture, containing ozone oxygen in ozonizer. Concentration of ozone in gas mixture was 1600 µg/l, in physiological saline 400 µg/ml. A cours of ozonetherapy included ozonized saline infusion, 200 ml a day each altimate day starting from the second day after surgery. Ozone concentration and the procedure frequency were worked out and confirmed in earlier gynecological, oncological and therapeutic researches.

Immunophenotype assay

Cell expression of CD3, CD4, CD8, CD16, CD20, CD95 and HLA-DR antigens of periferal blood mononuclear cells (PBMC) was quantified by flow cytometry. The PBMC was in staining buffer (PBS, pH 7.4 – 7.6) with corresponding antihuman monoclonal antibody for 30 minutes at 4ºC. The treated cells underwent the second antibody staining of FITC-goat antimouse immunoglobulin for labeling cells. Cells were washed twice with staining buffer, resuspend and fixed with fixation buffer (4% paraformaldehyde). Samples were analyzed using a FACScan (Becton Dickinson, San Jose, CA).

Soluble form of CD-antigens determination

Soluble CD38, CD50, HLA-I and HLA-DR were measured by a sandwich ELISA using corresponding capture antibodies coated at 100 µg/ml in saline buffer (pH 7.0) into 96-well ELISA plates at room temperature overnight. After 4 washes in PBS supplemented with 0.1% Tween-80 (PBS-T), serum was added at 1:1 dilution in PBS and incubated at room overnight. After 4 washes in PBS, captured soluble form of CD-antigens were detected with corresponding antibody at 100 µg/ml followed by color detection using HRP. All analyses were carried out in duplicate wells, and results were expressed in conditional units as a mean of soluble CD-antigens (CU/ml).

Lipid peroxidation and antioxidant system activity analysis

To determine lipid peroxidation and antioxidant system activity Fe^{2+}- and H_2O_2-induced biochemiluminescence assay was used. The essence of the method is that ions of metal with variable valency catalyzing decay of hydrogen peroxide lead to generation of free radicals (R', OH', RO, O'_2, RO'_2). Radicals cause free-radical oxidation. Recombination of RO'_2 results in forming of unstable tetroxide which decay and emit photon. Photon emission can be registered. To register biochemiluminescence serum of patient venous blood was used. Following chemiluminescence parameters were taken in account: Imax (mv)– maximum intensity of luminescence, it characterizes lipid peroxidation capacity of biological object; S (mv/30sec) – total luminescence during 30 sec describes discontinuity of the free-radical chain reaction; tg(-2)α – describes speed of free-radical process attenuation in serum ofblood.

The serum levels of lipid peroxidation products such as dien (DC) and triene conjugates (TC) were measured by reading of absorbance of lipid solution in methanol-hexane mixture at 233 and 275 nm. The level of the Shiff bases as the final products of peroxidation was measured by reading of absorbance at 420 nm (Fletcher).

Statistical Analysis

Data are expressed as means ± SD. The differences between the means of variables was analyzed by the Mann-Whitney U test.

Results and Discussion

Before surgery and any therapy the blood of the patients suffering from endometrial cancer contained significant higher levels of $CD16^+$, $CD20^+$ and $HLA\text{-}DR^+$ PBMC as well as lower levels of $CD3^+$ and $CD95^+$ cells relative to healthy controls ($p<0.05$) (Table 1).

Table 1 Relative quantity of PBMC in endometrial cancer patients before and after treatment

Cell population %	Healthy controls n=40	Control group n=26		Main group n=32	
		Before treatment	After treatment	Before treatment	After treatment
$CD3^+$	72,0±7,0	55,4±2,9*	51,7±2,6*	50,4±2,2*	53,5±2,9*
$CD4^+$	39,0±5,0	45,5±3,8	44,2±3,7	43,5±3,1	43,6±3,4
$CD8^+$	23,0±4,0	22,1±2,4	19,6±2,1	4,6±2,3	22,8±1,9
$CD16^+$	12,0±6,0	28,2±1,9*	24,3±2,2*	27,1±1,6*	20,0±1,5*▼
$CD20^+$	12,0±3,0	23,5±2,9*	27,5±2,6*	22,3±2,5*	27,8±2,8*
$CD95^+$	52,0±11,0	14,2±1,8*	19,8±1,7*	22,2±2,4*	25,6±3,1*
$HLA\text{-}DR^+$	14,0±7,0	30,4±3,1*	29,7±2,1*	30,7±3,1*	28,8±2,7*

** - significant differences in comparison with healthy controls (p<0.05)*

▼ - significant differences in comparison with pretreatment values (p<0.05)

n – number of patients

Before the treatment persons with hysteromyoma had significant higher levels of $CD8^+$, $CD20^+$ and $HLA\text{-}DR^+$ PBMC and lower $CD3^+$ and $CD95^+$-cells relative quantity in comparison with healthy controls (p<0.05) (Table 2). The differences in $CD4^+$ and $CD8^+$-lymphocytes concentrations were not established in cancer patients as well as in patients with hysteromyoma.

We found that persons with malignant and nonmalignant pathology had significant increased preoperative serum levels of sCD38, sCD95, sHLA-I and sHLA-DR molecules then in healthy controls. But the concentrations of sCD50 and sCD95 antigens were higher in patients with hysteromyoma relative to endometrial cancer patients (p<0.05) (Table 3, 4).

Standart therapy in endometrial cancer patients and uterine myoma patients was not attended significant changes in PBMC repertoire. In patients with endometrial cancer relative quantity of $CD16^+$ lymphocytes as well as serum levels of sCD38, sCD95, sHLA-I and sHLA-DR molecules went down to normal (p<0.05) only after ozonetherapy but not after standart treatment. We did not find significant differences in sCD50 concentration before and after any treatment.

Table 2 Relative quantity of PBMC in hysteromyoma patients before and after treatment

Cell population %	Healthy controls n=40	Control group n=22		Main group n=20	
		Before treatment	After treatment	Before treatment	After treatment
$CD3^+$	72,0±7,0	48,2±2,8*	48,6±2,4	50,2±2,6*	50,6±2,7
$CD4^+$	39,0±5,0	40,4±3,1	40,3±3,1	41,5±3,4	40,8±3,2
$CD8^+$	23,0±4,0	28,3±2,1*	27,6±2,3	30,2±2,4*	23,2±1,9*▼
$CD16^+$	12,0±6,0	20,6±1,8*	25,0±2,1	20,8±1,7*	21,8±2,7
$CD20^+$	12,0±3,0	28,2±2,6*	25,4±2,4	30,2±2,2*	20,3±2,4*▼
$CD95^+$	52,0±11,0	21,8±1,8*	29,8±1,7*▼	30,2±2,4*	24,4±2,8*▼
HLA-DR	14,0±7,1	28,0±2,1*	31,6±2,9	32,8±2,7*	29,6±2,8

** - significant differences in comparison with healthy controls (p<0.05)*

▼ - significant differences in comparison with pretreatment values (p<0.05)

n – number of patients

After ozonized saline infusion in hysteromyoma patients we found that the number of $CD8^+$, $CD20^+$ and $CD95^+$ PBMC went down to standard as well as the serum levels of sCD38, sCD50 and sHLA-DR molecules (p<0.05). The concentration of sHLA I also changed after ozonetherapy, but it went up to normal. Treatment with ozone led to further decreasing of the sCD95 level.

Table 3 Relative quantity of PBMC in endometrial cancer patients before and after treatment

Soluble molecule	Healthy controls n=40	Control group n=26		Main group n=32	
		Before treatment	After treatment	Before treatment	After treatment
sCD95	374,5±23,0	1470,0±191,0*	1239,0±184,0*	1163,4±161,2*	401,8±45,3▼
sCD50	353,8±65,0	83,8±85,1	494,0±50,5	432,8±75,3	278,3±69,7
sCD38	200,7±17,1	550,1±91,8*	300,2±79,4	410,2±43,4*	253,5±29,4▼
sHLA-I	1024,0±36,3	2888,0±251,2*	3014,0±280,1	2486,0±395,3*	1088,1±157,4▼
sHLA-DR	99,9±17,0	278,5±71,4*	201,9±75,6	175,7±30,3*	105,8±28,5▼

** - significant differences in comparison with healthy controls (p<0.05)*

▼ - significant differences in comparison with pretreatment values (p<0.05)

Values are expressed as U/ml. n – number of patients

Table 4 Relative quantity of PBMC in hysteromyoma patients before and after treatment

Soluble molecule	Healthy controls n=40	Control group n=22		Main group n=20	
		Before treatment	After treatment	Before treatment	After treatment
sCD95	374,5±23,0	568,4±59,7*	456,7±64,3	683,8±61,2*	506,6±46,5▼
sCD50	353,8±65,0	705,4±85,1*	443,7±5,5	511,3±75,3*	415,7±69,7
sCD38	200,7±17,1	373,0±71,8*	359,4±79,4	544,4±43,4*	228,8±29,4▼
sHLA-I	1024,0±36,3	389,8±36,2*	505,0±58,1*	286,5±57,1*	985,1±95,1▼
sHLA-DR	99,9±17,0	182,4±51,4*	165,2±55,6	256,8±27,3*	168,9±26,5▼

** - significant differences in comparison with healthy controls (p<0.05)*

▼ - significant differences in comparison with pretreatment values (p<0.05)

Values are expressed as U/ml. n – number of patients

The endometrial cancer patients had higher values of Imax and S parameters then in healthy controls before any therapy (p<0.05) (Table 5). After standart postoperative treatment the values of the indexes went up significantly whereas after ozonetherapy of cancer patients parameters Imax, S and tg(-2α) went down to standard. At the same time endometrial cancer patients of the control group had significant increased serum levels of DC, TC and Shiff bases relative to healthy controls (p<0.05). After the standard therapy the concentration of Shiff bases increased significantly. Cancer patients of the main group harbor higher TC and Shiff bases but not DC rates in comparison with healthy group (p<0.05). After ozonized saline infusion only DC serum rate went down significantly.

Table 5 Lipidperoxidation and antioxidant system parameters in patients with endometrial cancer

Parameter	Healthy controls n=40	Control group n=26		Main group n=32	
		Before treatment	After treatment	Before treatment	After treatment
Imax, mv	1,25±0,12	1,74±0,11*	2,13±0,11▼	1,79±0,10*	1,33±0,10▼
S, mv/30sec	14,0м1,5	18,42±0,74*	19,56±0,82*	19,37±0,81*	16,49±0,72*
tg(-2α)	0,28±0,02	0,324±0,001	0,51±0,01▼	0,346±0,01	0,257±0,01▼
Dien conjugates	0,165±0,05	0,18±0,03	0,22±0,03▼	0,15±0,02	0,157±0,026
Trien conjugates	0,04±0,01	0,06±0,01*	0,06±0,01	0,056±0,01*	0,03±0,01▼
Shiff bases	3,25±0,25	3,50±0,51	4,93±0,41▼	3,98±0,62*	3,82±0,62

** - significant differences in comparison with healthy controls (p<0.05)*

▼ - significant differences in comparison with pretreatment values (p<0.05)

n – number of patients

We found in the control group patients with hysteromyoma preoperative parameters of biochemiluminescence Imax, S and tg(-2α) were significant higher then in healthy controls (Table 6). After the standart postsurgical treatment index S went down to norm. There were not significant differences in values of parameters Imax, S and tg(-2α) in the main group of hysteromyoma patients relative to healthy controls. We did not found significant changes of these indexes after ozonetherapy.

Table 6 Lipidperoxidation and antioxidant system parameters in patients with uterine myoma

Parameter	Healthy controls n=40	Control group n=26		Main group n=32	
		Before treatment	After treatment	Before treatment	After treatment
Imax, mv	1,25±0,12	1,75±0,21*	1,45±0,10▼	1,46±0,20	1,59±0,31
S, mv/sec	14,0±1,5	17,50±0,75*	14,30±0,61▼	15,20±0,57	16,40±0,73
tg(-2α)	0,28±0,02	0,44±0,09*	0,40±0,08	0,33±0,07	0,40±0,06
Dien conjugates	0,165±0,05	0,27±0,04*	0,27±0,05	0,25±0,03*	0,26±0,03
Trien conjugates	0,04±0,01	0,133±0,02*	0,194±0,04▼	0,17±0,02*	0,09±0,001▼
Shiff bases	3,25±0,25	2,29±1,25	2,26±1,08	1,76±1,49	1,38±1,3

** - significant differences in comparison with healthy controls (p<0.05)*

▼ - significant differences in comparison with pretreatment values (p<0.05)

n – number of patients

Before any treatment in patients with uterine myoma DC concentration in serum of blood were significant higher in comparison with healthy controls (p<0.05). The Standart therapy didn't change this parameter.TC concentration was 3 time as much as in healthy controls and was rising after the standart therapy. We found that after ozonized saline infusion DC and TC serum levels came to standard. The differences of Shiff bases concentration in blood serum before and after any treatment were not established.

The results of the immunophenotype assay demonstrated that the endometrial cancer process as well as hysteromyoma process accompanied by different changes in PBMC repertoire. We found the endometrial cancer patients had normal levels of the majority of lymphocyte subpopulations before and after any treatment. Whereas the patients with uterine myoma contained increased rates of T-cell populations before the surgery. Ozonetherapy was attended $CD16^+$-cells number in cancer patients and rates of $CD8^+$and $CD20^+$-lymphocytes in myoma patients normalization. At the same time ozone treatment further decreased $CD95^+$-cells relative quantity. So we suggest that immune cells reduce their apoptotic activity in women with hysteromyoma. Changes in of cell quantity may reflect theraupetic efficiency of ozonetherapy in patients with endometrial cancer as well as hysteromyoma.

Ozonized saline infusion also normalized serum levels of soluble CD38, CD95, HLA-I and HLA-DR molecules in patients with endometrial cancer. Whereas in patients with hysteromyoma ozonetherapy decreased elevated preoperative serum levels of the molecules as well as increased to concentrations of sHLA antigen. Soluble molecules of membrane antigens can change functional activity of PBMC. Abundance or lack of them either activate lymphocytes or inhibit their functional activity [8]. Serum concentrations changes of these molecules in patients may be associated with pathogenetic mechanisms of hysteromyoma and endometrial cancer development. Normalization of soluble molecules level in serum after ozonetherapy suggests that ozone has influence on immune system of the patients.

The results of lipid peroxidation and antioxidant system assay demonstrate that in gynecological patients with endometrial cancer and hysteromyoma pro- and antioxidant balance is interrupted initially. Including ozonetherapy in standard treatment led to normalization of trien conjugates serum levels in nonmalignant patients as well as biochemiluminescence parameters and serum levels of lipid peroxidation products in cancer patients. This indicates the reparative action of ozone on the lipid peroxidation process and antioxidant activity of blood serum.

Conclusions

In summary, the results of the present study demonstrate that different forms of gynecological pathology are accompanied by significant changes in immune system as well as pro- and antioxidant systems. Intensity of these changes depends on the character of neoplastic process. Including ozonetherapy in complex postoperative treatment makes it possible to correct immune status and raise adaptation abilities of patients in the postsurgical period.

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