Experimental Research of Biological Effects of Ozone Therapy and Radiation Therapy in Cancer Animals

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Abstract

Malignant growth is known to disbalance the correlation between free radicals and antioxidant defence system. Is shown, that the introduction ozonated physiological saline as independent means, and in a complex with gamma-irradiation increases oxidizing reactions in tumor and suppresses it antioxidant protective properties; reduces the intensity glycometabolism in a tumor, that results in seen regressive morphological changes, namely: to increase of necrosis volume.

The pro-antioxidant balance of organism is restored, glycometabolism parameters and phagocytosis activity of polymorphonuclear leukocytes are normalized, level of endotoxemia is decreased. The assumption an opportunity of reduction of a used doze of an irradiation expresses.

Introduction

The mechanisms of intensifying of a radiation injury of a cell are connected to processes including development of free-radicals oxidation reactions in cellular membranes. In our opinion, the application of radial therapy in a complex with ozone therapy will result not only in rising selectivity of action of ionizing radiations based on oxygenous effect, but also intensifying dangerous free-radical environment of a tumor.

The idea to apply ozone in the treatment of neoplasia comes from the possibility to make use of the impaired metabolism of cancer cells.

Firstly, in malignant growth is known to disbalance the correlation between free radicals and antioxidant defence system. Tumor strains revel greater amount of natural antioxidants compared to that in the normal tissues. In neoplasia antioxidant defence system becomes exhausted, while in the tumor - antioxidant activity increases inhibiting free radicals and providing intense cellular proliferation (4,7). Hence, in this case ozone used to correct the cellular redox balance can be regarded as a measure capable to normalise free radical processes.

Secondly, cancer cells are distinguished by the respiratory disorder and increased capacity for intensive glycolysis. Consequently, the use of ozone as a powerful oxidiser can be regarded as a mighty agent capable to interfere into the competitive respiration /glycolysis relations within the tumor cell.

Thirdly, the genesis of malignant tumor is accompanied by inhibition of immune system.

Ozone effect revealed in enforcement of cellular and humoral immunity can be used as nonspecific defence of organism against the transformed cells (1).

The aim of the present work was to research of biological effects of influence of an ionizing radiation in a combination to ozone.

Materials and Methods

The experiment was done on 300 white male rats. The neoplasia was modelled through Lymphosarcoma (LS) clone (Institute of Experimental Oncology, Moscow) inoculation. The animals were subject to following actions: mono-radial influence, intra-abdominally, intra-and para-tumourally oxygen influence and gamma-irradiation; intra-abdominally, intra-and para-tumourally ozone influence and gamma-irradiation. The radiation dose of animals has made 0.2, 1.2, 2 Gy. Ozonated saline (OS) was used at concentration of ozone in gas mixture 100, 400, 900 and 3000 μg/l. Ozone was generated from medical grade oxygen, using electrical corona arc discharge in commercial ozone generator (Nizhni Novgorod, Russia).

On the first investigation phase the animals were divided in the following groups.

1- intact rats;
2- control group included tumor- implanted animals that did not receive treatment; 3- cancer animals with intra-and para-tumourally oxygen influence;
4- cancer animals with intra-and para-tumourally ozone influence;
5- cancer animals with only gamma-irradiation;
6- cancer animals with intra-and para-tumourally oxygen influence and ionizing radiation;
7- cancer animals with intra-abdominally oxygen influence and ionizing radiation;
8- cancer animals with intra-and para-tumourally ozone influence and ionizing radiation; 9- cancer animals with intra-abdominally ozone influence and ionizing radiation;
10- cancer animals with intra-and para-tumourally, intra-abdominally ozone influence and ionizing radiation;
11- cancer animals with intra-and para-tumourally, intra-abdominally ozone influence.

State of free-radicals and antioxidant processes. The generation or free- radicals activity of plasma and tumor tissue was investigated by chemiluminescence methods (model of Fenton reaction).

The products of lipid peroxidation: diene and trien cojugation (DC, TC), Shiff bases (SB) were measured in plasma. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP) and glutathione transferase (GT) were measured in erythrocyte hemolisates and tumor tissue homogenates. The preparation of hemolisate is described by Kosenko et al (7).

Glycometabolism activity was determined of amount of glucose, lactate, pyruvat.
Phagocytosis activity of polymorphonuclear leukocytes was detected by a method of luminol enhanced chemiluminescence. Chemiluminescence was measured on a BCHL-06 M biocounter luminometer (Nizhni Novgorod, Russia). The morphological and histological changes of tissues were observed.

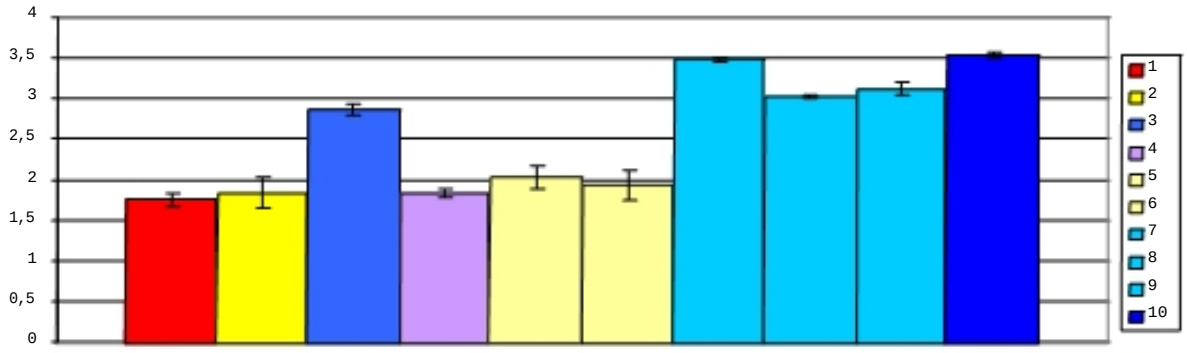
Results of all experiments were tested statistically ("Statistica- 5"). Data obtained are

expressed as arithmetic means and standard errors. An inferential statistical procedure was performed using the t-test. The level of significance was accepted with $p<0.05$.

Results and Measurements

The results of researches have shown, that the influence ozone results in essential changes of free-radicals processes and carbohydrate metabolism in tumor tissue.

First of all, introduction OS increased the free- radicals environment of a LS, oxidizing blockade of a tumor, initiates lipid peroxidation processes in neoplasia. To it testify increase of the contents of molecular products lipid peroxidation: DC, TC, SB and also increase of a tumor tissue homogenates chemiluminescence (Imax) (fig. 1,2,3).



1-control group included tumor- implanted animals that did not receive treatment; 2-cancer animals with intraand para-tumourally oxygen influence; 3-cancer animals with intra-and para-tumourally ozone influence; 4-cancer animals with only gamma-irradiation; 5- cancer animals with intra-and para-tumourally oxygen influence and ionizing radiation; 6- cancer animals with intra-and para-tumourally oxygen influence and ionizing radiation; 7-cancer animals with intra-and para-tumourally ozone influence and ionizing radiation; 8- cancer animals with intra-abdominally ozone influence and ionizing radiation; 9- cancer animals with intra-and para-tumourally, intraabdominally ozone influence and ionizing radiation; 10- cancer animals with intra-and para-tumourally, intraabdominally ozone influence.

Fig.1. Chemiluminescence activity of tumor tissue homogenatetes to oxygen, ozone and radiation influence.

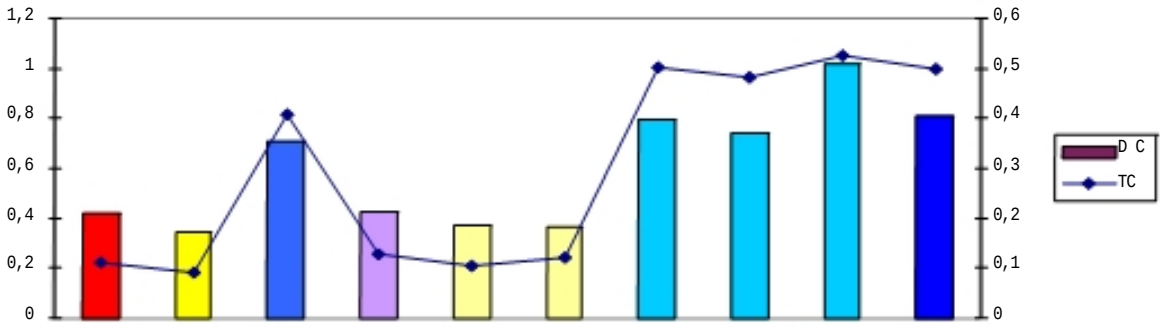


Fig.2. The contents of DC and TC in tumor tissue homogenatetes on mono- and combined influences of oxygenate, oxygenated saline solution and radiation.

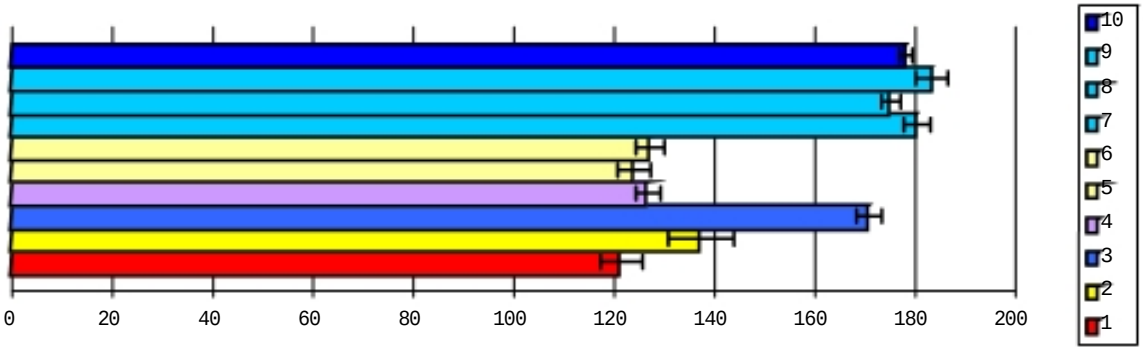


Fig.3. The content of SB in tumor tissue homogenatetes on mono- and combined influences of oxygenate, oxygenated saline solution and radiation.

Is observed significant decrease of activity of antioxidant enzymes SOD and CAT. Action of oxygen (oxygenated saline solution) and only of an irradiation do not result in changes the free- radicals status of a tumor. Namely: a chemiluminescence level, contents of LP molecular products, and also condition of components of antioxidant system of protection of a tumor in groups of the animals who have undergone to influences of oxygenated physiological solution and scale - radiation, in comparison with animals without influence, are not authentic (fig. 4).

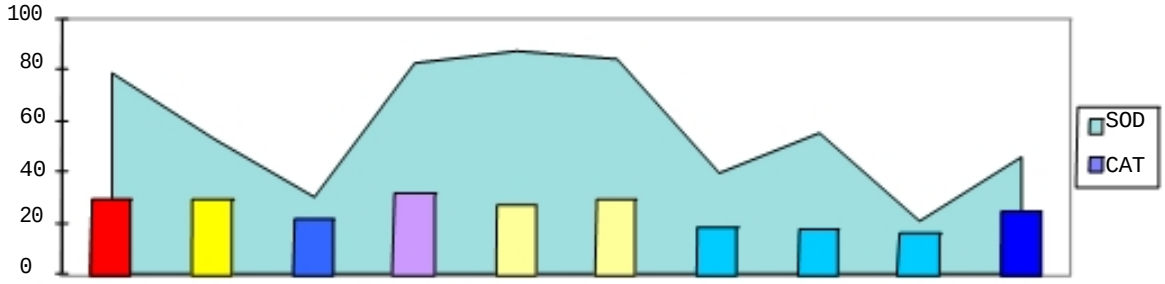


Fig. 4. Activity of antioxidant enzymes in tumor tissue homogenatetes on mono- and combined influences of oxygenate, oxygenated saline solution and radiation.

It is possible to regard the received significant reduction of the contents pyruvat and lactate in tumor tissue of experimental animals, with which entered OS, as result of an oppression of exchange processes LS (fig. 5).

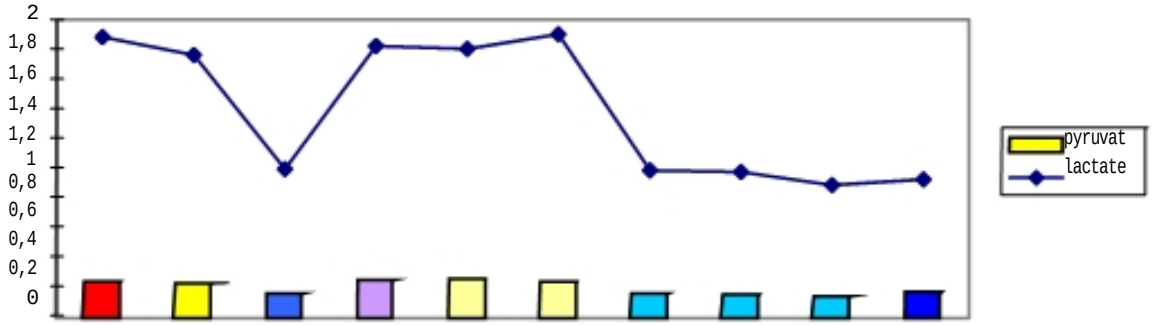


Fig. 5. The contents of pyruvat and lactate in tumor tissue homogenatetes on mono- and combined influences of oxygenate, oxygenated saline solution and radiation.

Convincing facts of OS anticancer effect are also results of morphological researches.
Microspecimen analysis revealed extensive destructive changes in tumours that could be explained by significant damage-producing effect of OS. The following disorders of blood circulation were especially evident: plethora, edema, haemorrhagic infiltration, thrombosis of microcirculatory bed. Alongside with dystrophic changes in parenchymatous cells and in microcirculatory bed there were found extensive sites of necrosis invading up to 50-75% of the whole tumour. It is important, that the direction necrosis in tumor is determined by localization of introduction OS: at para-tumourally influence the necrosis area is allocated from epydermis to a zone of tumor unit. At intratumourally introduction OS is observed necrosis of the central and transitive zones of tumor unit.

It is shown first of all in restoration of pro-antioxidant balance in blood of tumor -inoculated animals. As it is visible from the submitted results, mono- and combined influences OS in organism suppresses free- radicals oxidation, that the LP is expressed in decrease of the contents as molecular products and radical (fig.6, 7).

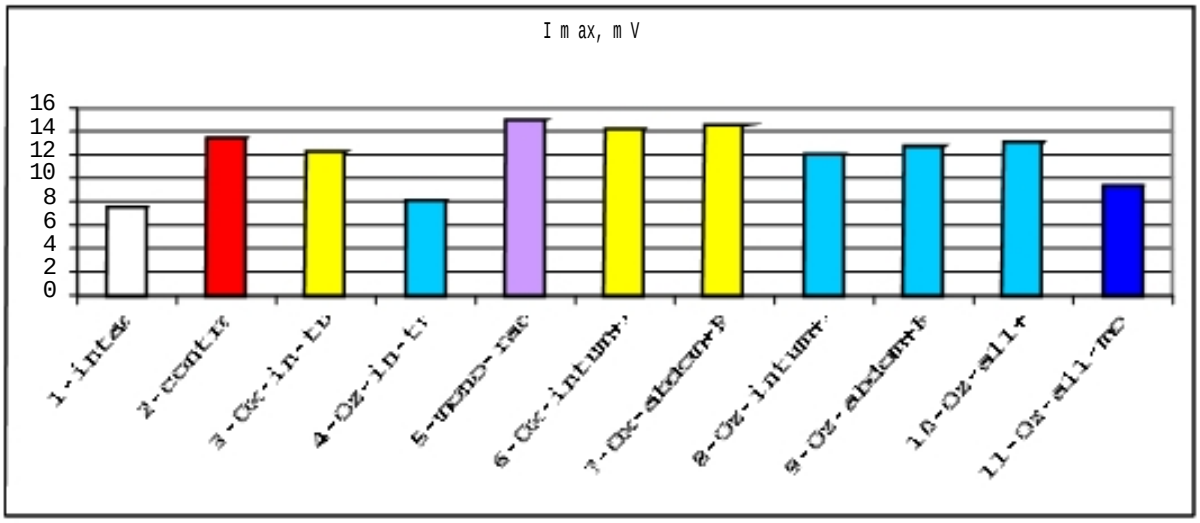


Fig.6. Chemiluminescence activity of rats plasma on mono- and combined influences of oxygenate, oxygenated saline solution and radiation.

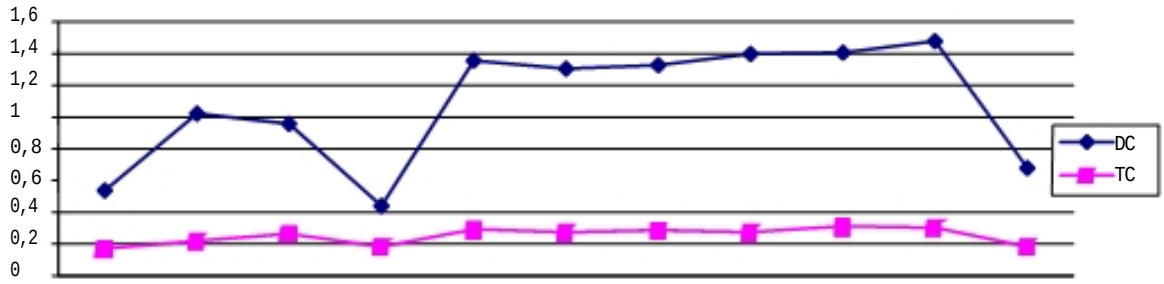


Fig.7. The contents of DC and TC in plasma of blood of experimental animals on mono- and combined influences of oxygenate, oxygenated saline solution and radiation.

The results of research have revealed increase of activity of enzymic components of antioxidat system of protection: SOD, CAT, glutathione -transferase and glutathione- peroxidase (tab. 1). In blood of the cancer animals who have undergone to influence OS, the increase of the

contents glutathione (tab. 1) is marked. Is established, that the application OS results in decrease of glycolysis parameters of blood animal (tab. 1).

Table 1. Antioxidant defese system, state of carbohydrate metabolism and endotoximia in blood of cancer animals after physico-chemical application.

	SOD	*CAT*	*GP*	*GT*	*GSH*	*Pyruvat*	*Lactate*	*Endotox. Index*
1	261,75±18,66	40,19±7,14	19,98±0,05	13±0,04	32,57±0,09	0,094±0,02	0,65±0,12	11,04±1,47
2	110,53±11,11*	17,24±1,91*	7,99±2,98*	7,11±0,21*	15,34±0,52*	0,158±0,04*	1,24±0,16*	36,65±8,71*
3	118,82±10,27*	19,27±1,35*	11,81±0,09*	6,35±0,93*	12,27±1,02*	0,149±0,14*	1,22±0,32*	36,73±6,5*
4	184,64±6,52**	33,93±2,71**	14,46±0,51**	12,47±0,39**	27,54±0,13**	0,108±0,02**	0,88±0,03**	25,8±2,93**
5	88,33±6,21*	18,38±1,02*	8,28±0,09*	8,51±0,47*	9,2±0,79*	0,133±0,004*	1,13±0,02*	34,65±7,1*
6	80,59±11,32*	19,04±2,11*	8,99±0,44*	8,03±0,25*	10,14±0,85*	0,139±0,07*	1,11±0,01*	33,48±5,03*
7	84,31±10,15*	18,53±1,28*	8,72±0,31*	8,42±0,19*	9,31±1,24*	0,137±0,03*	1,14±0,02*	34,18±5,14*
8	135,5±9,51**	25,03±3,21**	9,31±0,09*	9,58±0,31*	17,27±2,39**	0,121±0,02**	1,01±0,02*	31,57±1,42*
9	139,01±9,12**	23,14±2,44**	9,63±1,12*	10,87±0,24**	18,41±2,73**	0,119±0,01**	0,99±0,01**	27,14±1,89**
10	136,03±7,04**	27,8±3,62**	9,78±1,35*	11,02±0,63**	18,08±2,41**	0,108±0,01**	0,97±0,009**	26,18±2,24**
11	191,64±5,74**	30,54±2,17**	14,51±0,73**	12,59±0,41**	29,45±2,16**	0,106±0,01**	0,9±0,02**	20,58±3,49**

* - P < 0,05 as compared to intact group
** - P < 0,05 as compared to control group.

The results received at study of phagocytosis activity of blood showing about increase it (fig.8), probably, are explained, first, by disinfectant ozone effect at a local level, secondly, by system ozone action on immunity. The first assumption is connected that reactive oxygen species (ROS), which, on the one hand, act in organism at introduction OS, on the other hand, are formed in ozone process generates a cascade of ROS, represent additional a reserve of the biotoxic device. The second assumption concerns, in opinion V. Bocci to immunomodulation ozone effect (2, 3, 8). According the hypothesis that ozone can act a cytokine inducer by means of activation of nuclear factor kB (NF-kB), such as interferon (IFN-ß), tumor necrosis factor (TNF-alpha).

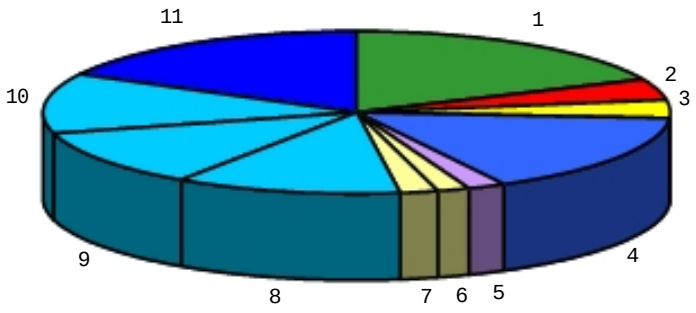


Fig. 8. Phagocytosis activity of polymorphonuclear leukocytes of rats in experimental groups.

Is established, that despite of development intensive of necrosis processes of a tumour caused by introduction in it OS, in blood of animals with LS the level endotoxemia is reduced (tab. 1). The received correlation interrelation between phagocytosis activity and index of endotoxemia ($r = -0,67$, $p < 0,05$), allows to assume, that the decrease endotoxemia is provided with amplification of phagocytosis activity.

The experiment on a survival has shown, that the introduction OS results in increase of life expectancy cancer animal on 30 %.

The anticancer effect of proves to be true by that fact, that at 80 % animal as a result of early introduction intra-abdominally ozonated saline in the concentration 400, 900 μg/l (for 7 days after tumor implantation) is observed damage of a tumor with complete normalization of biochemical parameters. However ozone concentration 900 μg/l in 17 % of animals causes metastasis of Lymphosarcoma.

That fact deserves the special attention, that combined ozone use and radiation has resulted in more significant amplification of free- radicals reactions in tumor tissue (tab. 1). However, at this influence in plasma of blood of experimental animals amplification a LP level is marked in comparison with intact animal (fig. 6, 7). It allows to assume, that further it is possible to reduce a used doze of an irradiation.

The results have confirmed our expectations: the combined of ozone action and scale - radiation at the reduced doze of an irradiation to a lesser degree promotes development of oxidizing stress in complete organism (fig.9).

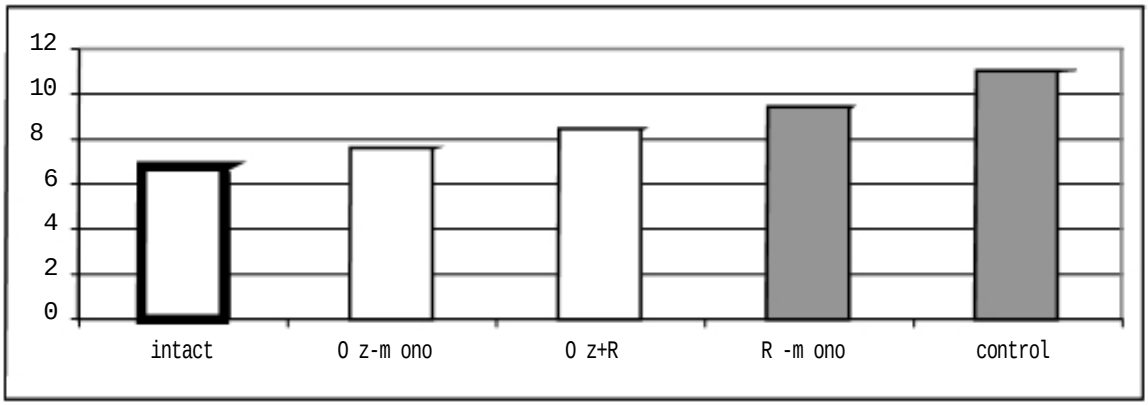


Fig. 9. Free-radical activity plasma of cancer rats with ozone and radiation at the reduced doze.

Positive dynamics of biochemical parameters, investigated by us, is a substantiation for the further researches of this approach.

Discussion

The oxygen tension of tissues/tumors treated with ionizing radiation has long been recognized as important factor influencing radiosensitivity and therapeutic response. The radiosensitizing

effects of oxygen are presumably mediated by free radicals and activated oxygen species. Because the radiosensitizing effect of oxygen saturates and plateaus at a pO_2 of about 30 torr, increases in the oxygenation of normal tissues theoretically should not alter radiation sensitivity significantly.

Therefore it is necessary or to lower an oxidative level in organism (hypoxia, antioxidants therapy (5)) or to raise concentration of radicals in a tumour (photodynamic, hyperthermia, hyperbaric oxygenation (4), treatment with inhalation of negative air ions (6), ozone therapy (3, 9, 10)).

The received data make it possible to suppose that OS local use produces antitumor effect.
Thus, use OS creates for a tumor an oxidative environment, oxidative stress results when the intracellular concentrations of reactive oxygen species, approaches crates neoplasia to limiting peroxidation conditions, causing them damage.

The expressed irreversible tumor damages of crates result, probably, in easing system action malignant neoplasia on organism of the owner. At the same time the glucose level increases in the blood of experimental animals. This proves that the organism managed to overcome and survive the state of hypoglycaemia.

OS results in the increase of phagocyte activity of immunecompetent cells in the blood of the animals. This type of non-specific immune-stimulation can be regarded as antitumor resistance.

The interest represents search effective ozone concentration for not only for local, but also for system action. In the latter case ozone can be used as a complex method with radiation, because both methods have the known free radicals mechanism of cell damage.

In this case it is important to estimate an initial level of free radicals in organism to avoid danger of conditions for metastasis.

So, the results of research allow to conclude, that ozone in high concentration at local introduction has antitumor activity, and in complex use with radiation therapy raises efficiency of anticancer therapy.

Conclusion

1. Intra-and para-tumourally oxygen influence of ozonated saline with ozone concentration in gas 3000 μg/l in tumor tissue results in decrease antioxidant activity, initiation of lipid peroxidation processes, decrease of glycolysis intensity, that is accompanied by regressive morphological changes Lymphosarcoma: substantial growth of necrosis volume.
2. The ozone concentration (900 μg/l) is revealed which at intra-abdominally introduction ozonated salime promotes damag primary tumor, however in 17 % of animals causes metastasis of Lymphosarcoma .
3. Combined use ozone therapy in the plan before radiation of preparation and reduced doze of ionizing radiation to a lesser degree promotes development of oxidizing stress in whole organism; and also results in normalization of a glycolysis, increase of viscosity of blood, luminol enhanced chemiluminescence of polymorphonuclear leukocytes, decrease of a internal toxicity level.

References

1. Bocci V. “Ozonization of bloood for the therapy of viral diseases and immunodeficiences. A hypothesis”, *Medical Hypotheses,* 39 (1): 30-4 (1992).
2. Bocci V., Luzzi E., Corradeschi F., Paulesu L. “Stadies on the biological effects of ozone : 5. Evaluation of immunological parameters and tolerability in normal volunteers receiving ambulatory autohaemotherapy’’, *Biotherapy* 7: 83-90 (1994).
3. Bocci V. Ozone as a bioregulator. Pharmacology and toxicology of ozonetherapy today *J. Biol. Regul. Homeost. Agents*., 10(2-3): 31-53(1996)
4. Fuchs J., Thiele J. “The role of oxygen in cutaneous Photodynamic Therapy”, *Free Rad. Biol & Med.*,24 (5): 835-847 (1998).
5. Fuchs J.” Potenntials and Limitations of the Natural Antioxidants RRR-Alpha-Tocopherol, L-Ascorbic acid and in Cutaneous Photoprotection”, *Free Rad. Biol & Med.* 25 (7): 848-873 (1998).
6. Kondrashova M.N., Grigorenko E.V., Tikhonov A. N. “The Primary Physico-Chemical Mechanism for the Beneficial Biological/Medical Effects of Negative Air Ions”, *J.IEEE Transactions on Plasma Science*, 28 (1) :230-237 (2000).
7. Kosenko E.A., Kaminsky Y.G., Stavrovskaya I.G. “The stimulatory effect of negative air ions and hydrogen peroxide on the activity of superoxide dismutase”, *FEBS Lett.,* 410:309-317 (1997).
8. Ryter S.W., Tyrrell R.M. “Singlet Molecular oxygen: a Possible Effector of Eukaryotic Gen expression”, *Free Rad. Biol & Med.,* 24 (9):1520-1534 (1998).
9. Viebann R. The biochemical process underlying ozone therapy *Ozonachrichter.,* 4:18-30 (1985).
10. Viebahn-Hansler R. *Ozone-handbook*. (Theraploose-leaf edition, 1997). p. 400.