TRIAL PROTOCOL

(confidential)

Title: INFLUENCE OF BIO-NORMALIZER ON TOXICITY INDUCED BY THERAPEUTIC IRRADIATION

Phase III

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1.0 Checklist for Patient Eligibility and Necessary Information

Age between 2-15 years.
Cytological evidence of acute lymphoblastic leukemia with clinical neuroleukemia or in relapse.
Cytological evidence of acute myeloblastic leukemia.
Evidence of measurable disease.
Recovered from toxic effects after previous chemotherapy.
Cranial irradiation.
HIV infection
Bone marrow total cytosis > 50000, blast cells < 5%.
Peripheral absolute neutrophil count > 500/mm^3
Platelet count > 100000 mm^3
Bilirubin < 2.0 mg/dL, SGPT < 500 U/L
Normal coagulation screen
Creatinine < 2.0 mg/dL.
Normal urinanalysis.
Informed consent explained to and signed by patient/guardian.

2.0 Objectives and Rationale

2.1 To determine the toxicity of cranial irradiation following the polychemotherapy of pediatric patients with acute lymphoblastic leukemia with clinical neuroleukemia or in relapse and with acute myeloleukemia.
2.2 To determine the influence of Bio-normalizer (BN) on adverse effects of therapeutic irradiation.
2.3 To determine the effect of BN on the biochemical and cytological parameters of spinal fluid and on the free radical status of a child's organism.

3.0 Background

The intensive worldwide researchs of free radicals of the past 15 years have showen that oxygen free radicals play an important role in development of various pathologic processes such as inflammation, tumors, allergy, degenerative disorders, age- and environment-induced damages. It is now well known that an unusually large amount of free radicals can be formed in pediatric patients with many blood diseases (leukemias, aplastic anemias, hemolytic anemias, thalassemia, etc.). Moreover, there is a large body of evidence that the action of many antitumor drugs, for example anthracycline antibiotics and bleomycin, as well as therapeutic irradiation is mediated by oxygen radicals. At the same time, an excess of these toxic species in an organism may be an origin of serious deleterious effects in normal cells and tissues. Therefore, it is believed that oxygen radicals are responsible for the adverse side effects of these drugs and of other therapeutic treatments.

For example, in order to prevent neuroleukemia development, therapeutic cranial irradiation was applied to the patients with different hemoblastosis after previous polychemotherapy. (Neuroleukemia is known to be one of the most dangerous life-threatened features of leukemias). However, the irradiation of the child head resulted in mild or strong encephalopathy developing 4-8 weeks after irradiation. Such a postponed toxicity may be due to free radical damage of endothelium of brain vessels and developing edema or possibly mielin degradation.

Under physiological conditions, the level of organism's oxygen radicals is controlled by powerful antioxidant systems including enzymes (SOD, catalase, glutathione peroxidase, etc.) and low-molecular antioxidants such as glutathione, ascorbic acid, and α-tocopherol. If endogenous antioxidant systems deteriorate as a result of permanent oxidative stress, the intervention of highly toxic agents, or the mulfunctions of cellular organelles, a dangerous elevated level of oxygen radicals may be rapidly achieved.

We supposed that BN administration can significantly improve the free radical status of patients by suppressing damaging post-effects of irradiation and diminishing the risk of the development of encephalopathy. Indeed, it has been shown that BN is a hydroxyl radical scavenger (Santiago, et al., 1991) and powerful inhibitor of the release of highly reactive oxygen species by phagocytes (Osato, et al., 1994). In addition, it has been recently found (Korkina, et al., 1994) that BN is able to enhance the level of reduced glutathione (one of the most important endogenous antioxidants) and to stimulate the SOD activity. On these grounds, n this pilot study we were testing the efficiency of BN in prevention of irradiation-induced brain pathology and were studying the possibility of normalizing free radical processes in blood and spinal fluid of patients during and after cranial irradiation.

4.0 Patient Eligibility

4.1 All the patients from 2 to 15 years of age previously treated with multidrug chemotherapy, who at the risk of neuroleukemia development and need therapeutic cranial irradiation in dose more than 18 Gy.

4.2 Cytological evidence of diagnosis of lymphoblastic leukemia with initial neuroleukemia or in relapse; acute myeloleukemia.

4.3 Patients must have recovered from the toxic effects of all prior chemotherapy.

4.4 Patients must have adequate bone marrow function (defined as a total cytosis > 50000/ml and the blast cells content < 5%).

4.5 Patients must have normal parameters of peripheral blood (defined as a peripheral absolute neutrophil count (ANC) of > 500/mm^3, platelet count of > 100000/mm^3, and hemoglobin content > 10 g/dL).

4.6 Patients must have _____ .

5.0 Treatment plan

Patients will be given 1 sache of BN 3 times a day during one month just after the completion of irradiation treatment.

6.0 Patient Evaluation

7.1 A complete history and physical examination with documentation of measurable disease is necessary for admission to the study.

7.2 *Other studies. Sequences.*

	Before BN treatment	After BN treatment
Physical examination	x	x
Total blood cound including RBC, WBC, platelets, blasts, IIb	x	x
Biochemical blood analysis (bilirubin, urea, total protein, ALT, AST, electrolytes, iron)	x	x
Urinalysis	x	x
Spinal fluid analysis (cytosis, total protein, cytopreparate)	x	x
The determination of free radical status in blood (the intensity of leukocyte chemiluminescence, the content of glutathione, the activity of SOD, etc.)	x	x

Ethics Commission

Pursuant to the Declaration of Helsinki, the present study program was submitted to an independent Ethics Commission of the Russian Institute of Pediatric Hematology, Moscow, for review. The decision of this Ethics Commission must be presented in writing to the study director and organizing monitor. Any recommendations and requirments made by the Commission must be incorporated into the study schedule.
The start of the study is dependent on a positive decision by the Ethics Commission.

Patient Information and Consent

The clinical investigator must instruct the patients about the nature, importance and scope of the trial. He (she) must obtain and countersign the written declaration of informed consent of each patient before his/her participation in the study. A copy of the declaration of informed consent must be handed out to every study participant.

Signatures
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Part finished p. 6 - 15
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CLINICAL TRIAL

INFLUENCE OF BIO-NORMALIZER ON NEUROTOXICITY INDUCED BY THERAPEUTIC IRRADIATION

(Pilot study)

FINAL REPORT

1. CHILDREN

Chidren were suffered from acute lympholeukemia (11 children) and acute myeloleukemia (19 children). All of them have been examining and treating at the Russian Institute of Pediatric Hematology. General characteristics of the children studied are presented in Tables 1-6. Chidren taking part in the clinical trial were divided into 6 randomized groups:

Group A: 4 children suffered from acute lympholeukemia were given standard chemotherapy for 1 month and then were subjected to cranial irradiation in dose 12 Gy (Control 1).

Group B: 7 children were subjected to the same treatment as the children of Group A, but in addition they were given 1-3 saches of BN a day for two weeks before irradiation.

Group C: 3 children suffered from acute myeloleukemia were given standard chemotherapy plus cranial irradiation in dose 18 Gy (Control 2).

Group D: 3 children were treated as those of Group C, but in addition they were given 1-3 saches of BN a day for 1 month after irradiation treatment.

Group E: 7 children suffered from acute myeloleukemia were under the intensive course of chemotherapy (Control 3).

Group F: 6 children were treated as those of Group E, but in addition they were given 1-3 saches of BN a day for 1 month after the chemotherapy completing.

During the clinical trial, all traditional hematological and biochemical analyses essential for the evaluation of efficacy of the chosen treatment and the main living functions were performed several times. They were a full blood count (the hemoglobin content, differential cell count, hematocrit, cell size distribution), a special histologial analysis of the blood and bone marrow smears, a total protein, billirubin, and creatinin contents, and ALT and AST activities. For measurement of the organism's oxidative stress, the intensities of spontaneous and latex-activated luminol-dependent chemiluminescence in isolated leukocytes, CuZnSOD activities in erythrocytes, leukocytes, plasma, and spinal fluid, and MnSOD activity in leukocytes were determined. The corresponding analytical techniques are given below. There were no drop-outs in the clinical trial due to the adverse effects of drug administraion.

2. EXPERIMENTAL METHODS

2.1 Blood Samples

Venous blood was drawn by disposed syringes early in the morning before breakfast into three tubes: one of them was with 20 U/ml heparin, second was with sodium citrate and the third was without any anticoagulant. 1.5 ml blood was collected into the first tube, 0.5 ml was collected into the second tube, and 9 ml was collected into the third one. Serum was obtained from the 9 ml blood sample by centrifugation and then used for biochemical assay. The 0.5 ml blood sample was used for differential cell count, and the 1.5 ml blood sample was used in CL, SODs, and glutathione assays.

2.2 Preparation of Leukocytes

The blood samples (1.5 ml) were anticoagulated with 0.2 ml of heparin (20 U) in Hanks' balanced salt solution (HBSS) and sedimented with 1.5 ml of dextran-metrizoate mixture (50 ml 6.2% dextrane/20 ml 38% metrizoate) at 25°C for 30 min. The cell-rich supernatant was centrifuged at 150xg for 10 min. Cell pellets were washed twice in HBSS. The final suspension of 2-3x10^6 leukocytes was prepared in the cultural medium 199. The cells were counted with a microscope, and their viability was assessed by exclusion of 0.1% trypan blue dye. Cell differential count was confirmed in the cell smears using Giemsa staining.

2.3 Chemiluminescence Analysis

Luminol-dependent chemiluminescence was measured on a LKB luminometer mod. 1251 (Sweden). Leukocyte suspension (20 µl, 2x10^6 cells/ml), luminol (50 µM, final concentration), and 0.85 ml of HBSS were mixed in the 1 ml cuvette at 37°C. After 5 min, the 0.1% suspension of latex (Sigma) in 0.95% NaCl solution was added, and the light emission was recorded continuously. The intensity of spontaneous CL and the difference between the maximal values of the cellular CL response to latex and of spontaneous CL were measured.

2.4 Measurement of Glutathione Metabolism in Red Blood Cells

Erythrocytes were obtained after blood sedimentation on dextran-metrizoate gradient and washed twice in the cold phosphate buffer, pH 7.2. The content of reduced glutathione was determined by the Beutler method[6].

2.5 Measurement of SODs and catalase activities

Superoxide dismutase (CuZnSOD) activity was determined by the adrenaline method[7], in which the rate of superoxide production was measured by lucigenin-amplified CL[8]. Heparinized venous blood (0.2 ml) was hemolyzed with ice-cold water. Lysate was added to the equal volume of ethanol-chloroform mixture (1:1 v/v) and centrifuged at 1500 x g for 30 min. Protein content in supernatant was determined by the Lowry method[9]. To measure the total SOD activity, 50 mL top clear supernatant was added to carbonate buffer (pH 10.2, a total volume of 900 mL) containing EDTA (100 mM) and lucigenin (100 mM) in the polysterene CL cuvette ,and the level of basal CL was registered continuously. Reaction was started by adding 50 mL adrenaline (100 mM) through the dispenser . CL light sum for 5 min was recorded and compared with that of a control sample (50 mL water:ethanol:chloroform solution (2:1:1 v/v/v)). The total SOD activity was calculated from calibration curve using commercial SOD as a standard and expressed as U/mg protein. For the determination of MnSOD activity, CuZnSOD was inhibited by the addition of NaCN (4 mM) to the supernatant. After that, the CL measurement of MnSOD activity was performed as described above. CuZnSOD activity was estimated as a difference between the total and MnSOD activities.
Catalase activity was determined in the same lysates according to Aebi[10]. Enzyme activity was expressed as units per mg protein.

2.6 Statistical Analysis

The clinical trial was a randomized open study on 30 children with daily oral doses of Bio-normalizer. Student's t-test for mean values was used for evaluation of significance between different experimental groupes. Paired significance test was used for connected

values obtained before and after the clinical trial. 5% significance level was assumed. Results were presented as mean ± SEM.

3. RESULTS AND DISCUSSION

The examination of children by pediatricians during and after the completion of the clinical trial has shown that there were no toxic side effects, allergic reactions and other adverse events during the BN administration. Biochemical analyses confirmed these observations. Pediatricians concluded that there was a good tolerability to the BN application. More than that, in accord with previous findings, there was significant beneficial effect of BN administration on the liver functions (Table 7). As is seen from this Table, even short-term BN administration statistically significantly improved liver enzyme activities (ALT and AST) that reflected the activation of the reparative processes in liver which certainly was damaged during chemotherapy.

3 of 4 children (75%) of Group A exhibited severe neurotoxic symptoms after irradiation exposure. One of them died of the relapse of ALL (Table 8). At the same time, only 2 of 7 children (28%) of Group B treated with BN before irradiation cycle exhibited moderate encephalopathy, all of them achieved a remission and until now they are alive (Table 8).

The more strong beneficial effect of BN application was found in a comparative study of the children from Groups C and D (Table 9). All children of Group C (100%) exhibited a moderate encephalopathy, and no one of Group D (0%) treated with BN after the irradiation had any neurotoxic symptoms.

Comparison of children from Groupes E and F shows that in the control Group two of 7 children (28%) died, being non-respondable to the applied chemotherapy. In contrast, children of Group F who were given BN after chemotherapy, exhibited no relapses (0%), and all of them are alive (Table 10). However, BN did not affect the agranulocytosis duration. An average duration of the drug-induced aplastic syndrome was 17.6 days in the

control Group and 33 days in the experimental Group E (Difference was statistically insignificant). These results could be explained in terms of cytokine production activation during BN administration. It has been previously shown in the *in vitro* experiments that BN can stimulate γ-interferon and α-TNF production by monocytes and macrophages. These particular cytokines are capable of suppressing the bone marrow cell proliferation. That may cause a slight prolongation of the symptomes of the drug-induced aplasia.

One of the causative reasons of the toxic effects induced by chemotherapy and therapeutic irradiation may be the overproduction of oxygen free radicals in an organism. It is now well established that these very reactive species are able to damage DNA, proteins, lipids, polysaccharides, and various biological structures including neurons and myelin. Due to that, the disturbances in the hematopoietic tissues, the immune system, and the central and peripheral nervous systems occur. There are at least four major pathways of suppressing free radical processes and reducing the state of oxidative stress:

a) scavenging of free radicals by antioxidants and oxygen radical scavengers;

b) the stimulation of the activities of the endogenous antioxidant systems;

c) suppressing the activities of the enzymes catalyzing the oxygen radical generation;

d) chelating and inactivating active ions of transition metals, which catalyze the production of the most aggressive hydroxyl and hydroxyl-like radicals.

There is a body of evidence that Bio-normalizer can influence free radical processes in organism due to its significant hydroxyl radical scavenging activity [ ]. Furthermore, it was shown by us in the previous study that BN enhanced CuZnSOD activity in the macrophages [ ] and to some extent inhibited the NADPH-oxidase of neutrophils and macrophages. It could be suggested also that BN possessed a weak chelating activity because it contains a large amount of polysaccharides. Therefore, BN can interfere with endogenous free radical processes by different metabolic pathways.

Until now, there were no data whether BN can affect glutathione metabolism. Glutathione is one of the major blood antioxidant responsible for the inactivation of various

oxidants formed during drug and other xenobiotics metabolism or as a result of the environmental hazard. In present work it was found that irradiation and chemotherapy substantially decreased the level of reduced glutathione in the erythrocytes, while BN given before or after the irradiation treatment increased its level up to the normal values (Table 11). At the same time, BN did not affect the level of glutathione when it was given after chemotherapy (Group F). These data allowed us to suggest that a very strong protocol treatment of lympho- and myeloproliferative malignancies caused a suppression of glutathione system, that can be an origin of the oxidative stress-induced deleterious effects of therapeutic irradiation and chemotherapy. BN can prevent such deleterious effects of irradiation but not chemotherapy by inducing glutathione synthesis and/or reducing oxidizing glutathione. What is a reason for the diverse effects of BN on irradiation and drug-induced glutathione depletion? That may be discovered only in the course of further studies.

It was found that BN administration before and after irradiation induced the CuZnSOD activity in children' leukocytes and the catalase activity in erythrocytes (Table 12). There were no significant changes in the erythrocyte CuZnSOD activity (Table 13). Surprisingly, leukocyte MnSOD activity was suppressed by BN administration at its high level and was stimulated by BN at low level (Table 14). These results may be explained in terms of the capacity of BN to increase the organism potency to be adapted to oxidative stress. There is growing body of evidence that one of the genetically regulated properties of mammalian cells is the abilty to be adapted to oxidative stress. Some proteins exhibit increased expression during such an adaptation, among of them are antioxidant enzymes, DNA repair enzymes, DNA damage-specific gene products, heme oxygenase, heme binding proteins, etc.[11,12]. Oxidative stress can affect enzymes involved in intracellular signal transduction that in turn lead to blocking cell proliferation and activating apoptosis in bone marrow stem cells[13]. Moreover, it is now well established that strong prooxidants such as hydroxyl radicals and peroxynitrite are involved in carcinogenesis inducing protooncogene

expression, influencing tumor initiation and promotion[14,15]. On the basis of the recognized role for reactive oxygen species in tumor initiation and promotion, it has been hypothesized that antioxidant enzyme genes possess anti-oncogenic properties[16]. A decrease of the activity of antioxidant enzymes may enhance the oxidative stress, which allows the tumor to arise and develop. Therefore, it is important that BN administration allow to maintain the normal activities of main antioxidant enzymes such as CuZnSOD, MnSOD and catalase. It seems that the target for the BN stimulating activity is predominantly blood circulating monocytes and tissue macrophages.

It should be stressed that reactive oxygen species do not only exhibit deleterious effects but also possess very important physiological properties. Thus, it has been shown many times that superoxide and hydrogen peroxide can defend an organism against bacterial infections, play a role as adaptogenes inducing endogenous free radical defense systems, and stimulate growth, maturation, and differentiation in a variety of mammalian cells[17]. While hydroxyl radicals are known as the most aggressive agents capable of initiating the irreversible damage, superoxide and hydrogen peroxide can interfere with physiological processes and regulate them. Therefore, deficiency of these physiologically important reactive oxygen products can suppress cell growth and maturation[17,18]. As is seen from Table 15, BN administration markedly increased spontaneous and latex-activated luminol-dependent CL in the blood leukocytes of the most of patients. This effect may be easily interpreted taking into account that due to agranulocytosis (a granulocyte deficiency) after the intensive chemotherapy, the main source of the essential oxygen free radicals became blood monocytes. BN is known as a potent activator of monocyte-macrophage system, and therefore it can stimulate the blood monocytes to produce more radicals in order to compensate the granulocyte deficiency.

Table 1. General characteristics of patients

Group A (control 1)

Number	Name	Diagnosis, group of risk	Sex	Age, years
1	Ganin	ALL, mild	M	6
2	Kopylov	ALL, moderate	M	2
3	Sitnikov	ALL, mild	M	4
4	Dovlatyan	ALL, moderate	F	2

Group B: BN for 2 weeks before the 12Gy irradiation

Number	Name	Diagnosis, group of risk	Sex	Age
1	Kchabalaeva	ALL, mild	F	5
2	Kulchenko	ALL, moderate	M	5
3	Malofeeva	ALL, moderate	F	16
4	Bandura	ALL, mild	M	4
5	Vasinov	ALL, moderate	M	4
6	Frolov	ALL, mild	M	4
7	Laptev	ALL, moderate	M	8

Group C (control 2)

Number	Name	Diagnosis, group of risk	Sex	Age, years
1	Sedov	AML, M5, II	M	11
2	Knyazeva	AML, M2, I	F	15
3	Gerasimova	AML, M2, I	F	3

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Group D : BN for 1 month after the 18 Gy irradiation

Number	Name	Diagnosis, group of risk	Sex	Age, years
1	Bubnov	AML, M2, I	M	13
2	Mormel	AML, M5,II	F	5
3	Sushkov	AML,M4,II	M	8

Group E (control 3)

Number	Name	Diagnosis, group of risk	Sex	Age
1	Smirnov	AML, M2,I	M	12
2	Mishina	AML, M5, II	F	6
3	Kotova	AML, M1, I	F	15
4	Sidenko	AML, M5, II	M	11
5	Bazylina	AML, M4, II	F	10
6	Sautina	AML, M4, II	F	10
7	Gerasimova	AML, M2, I	F	3

Group F: BN after the intensive chemotherapy

Number	Name	Diagnosis, group of risk	Sex	Age, years
1	Shurupov	AML, M5a, II	M	8
2	Zmyrko	AML, relapse	F	16
3	Ivanova	AML, relapse	F	13
4	Sazonova	AML, M1, II	F	10
5	Verlina	AML, M5a, II	F	6
6	Petrov	AML, M5a, II	M	6

Table 7. ALT and AST activities in the blood plasma before and after the BN administration.

Group B

Number	ALT		AST	
	before	after	before	after
1	261	22.5	176	23.5
2	35.7	41.4	41.1	46.2
3	150	53.5	64.4	24.8
4	200	42.7	93.1	31.6
5	106	76.8	58.2	31.0
6	29.8	31.8	34.8	41.1
7	200	142	99.3	62.8
$\bar{M}$	140.4	58.7	81	37.3

Group D

Number	ALT		AST	
	before	after	before	after
1	82	82	48	48
2	29	29	31	31
3	116	100	78	60
$\bar{M}$	75.7	70.3	52.3	46.3

Group F

Number	ALT		AST	
	before	after	before	after
1	68	116	60	68
2	88	30	119	42
3	92	150	73	87
4	316	154	168	127
5	93	78	105	77
6	78	69	103	61
$\bar{M}$	122.5	99.5	104.7	77

Table 8. The clinical effect of BN administration in patients of Group B comparing with control 1

Group A (control 1)

Number	Adverse effects	Encephalopathy	Remission
1	no	0	+
2	yes	2.0	+
3	yes	1.5	+
4	yes	2.5	-, ex. let.

Average score 1.5

Group B

Number	Adverse effects	Encephalopathy	Remission
1	no	0	+
2	fever	1.0	+
3	no	1.5	+
4	fever	0	+
5	no	0	+
6	no	0	+
7	no	0	+

Average score 0.35

Table 9. The clinical effect of BN administration in patients of Group D comparing with control 2

Group C

Number	Adverse effects	Encephalopathy	Remission
1	no	1.5	+
2	yes	2.0	+
3	yes	2.0	+

Average score 1.9

Group D

Number	Adverse effects	Encephalopathy	Remission
1	no	0	+
2	no	0	+
3	no	0	+

Average score 0

Table 10. The clinical effect of BN administration in patients of Group F comparing with control 3

Group E

Number	Adverse effects	Aplastic syndrome, days	Remission
1	no	16	+
2	no	20	+
3	no	17	+
4	no	19	+
5	no	14	+
6	no	15	- ex. let.
7	no	22	- ex. let.

Average value 17.6

Group F

Number	Adverse effects	Aplastic syndrome, days	Remission
1	no	15	+
2	no	35	+
3	no	71	+
4	no	16	+
5	no	29	+
6	no	32	+

Average value 33.0

Table 11. Reduced glutathione content (GSH) in the erythrocytes before and after the BN administration and before and after irradiation.

Group B

Number	BN		Irradi ation	
	before	after	before	after
1	6.9	10.0	10.0	9.4
2	7.3	8.2	8.2	5.6
3	10.5	10.1	10.1	8.4
4	10.2	10.8	10.8	8.7
5	10.1	10.2	10.2	9.7
6	5.9	6.6	6.6	6.2
7	5.6	7.4	7.4	6.5
$\bar{M}$	8.1	9.0	9.0	7.8

Group D

Number	Irradi ation		BN	
	before	after	before	after
1	7.8	8.8	8.8	9.0
2	6.9	4.9	4.9	6.8
3	7.1	6.3	6.3	7.8
$\bar{M}$	7.3	6.7	6.7	7.9

Group F

Number	Chemothe rapy		BN	
	before	after	before	after
1	6.9	7.0	7.0	7.9
2	7.5	7.1	7.1	7.9
3	7.0	7.5	7.5	7.3
4	7.9	6.9	6.9	6.7
5	11.8	11.9	11.9	10.4
6	6.8	7.3	7.3	11.9
$\bar{M}$	7.95	7.95	7.95	8.7

Table 12. SODs and catalase activities before and after BN administration.

Group B

Number	Leukocyte CuZnSOD		Leukocyte MnSOD		Erythrocyte catalase	
	before	after	before	after	before	after
1	333	810	116	455	17	37
2	304	380	246	76	24	42
3	176	250	250	66	29	32
4	132	112	44	62	32	48
5	141	205	41	122	26	39
6	61	42	46	14	37	36
7	234	382	525	26	25	30
$\bar{M}$	197.3	311.6	181.2	117.3	27.2	38

Group D

Number	Leukocyte CuZnSOD		Leukocyte MnSOD		Erythrocyte catalase	
	before	after	before	after	before	after
1	326	442	276	509	n.a.	n.a.
2	245	455	177	531	n.a.	n.a.
3	140	304	70	61	26	38
$\bar{M}$	237	400.3	174.3	367		

Group F

Number	Leukocyte CuZnSOD		Leukocyte MnSOD		Erythrocyte catalase	
	before	after	before	after	before	after
1	31	304	28	26	17	35
2	59	317	68	26	24	32
3	88	414	49	242	n.a.	n.a.
4	104	152	15	66	n.a.	n.a.
5	129	52	129	37	21	46
6	80	80	80	50	14	27
$\bar{M}$	81.8	219.8	61.5	74.5	19	35

Table 13. Luminol-dependent CL intensities before and after BN administration

Group B

Number	Spontaneous		Latex-activated	
	before	after	before	after
1	1222	154	11156	285
2	386	203	8237	8707
3	2279	220	13308	12737
4	792	2168	5472	13439
5	630	741	3167	6935
6	360	2012	3257	11821
7	929	118	5714	634

Group D

Number	**Spontaneous**		**Latex-activated**	
	before	**after**	**before**	**after**
1	**138**	**425**	**4070**	**7928**
2	**296**	**321**	**10112**	**1175**
3	**1016**	**1285**	**14371**	**55869**

Group F

Number	Spontaneous		Latex-activated	
	before	after	before	after
1	72	120	1496	2700
2	482	6	1841	10
3	71	433	1434	4425
4	810	9	7840	24
5	400	1105	3640	7995
6	4860	762	51300	16269

4. CONCLUSIONS

1. BN administration significantly suppressed encephalopathy induced by therapeutic irradiation.

2. BN administration improved the liver functions impaired during chemotherapy and therapeutic irradiation.

3. BN administration restored the level of reduced glutathione which was decreased after the irradiation treatment.

4. BN administration stimulated activities of the main antioxidant enzymes such as leukocyte CuZnSOD and erythrocyte catalase.

5. BN administration stimulated oxygen radical production by blood monocytes compensating the granulocyte deficiency.

6. BN administration did not exhibit any side toxic, allergic, and other adverse effects on children.

7. BN administration did not change the duration of aplastic symptomes induced by chemotherapy.

8. BN can be promising medicine as a supportive care for cancer patients after intensive course of chemotherapy and irradiation . It can diminish their neurotoxic and other adverse effects and maintain the high quality of life of these patients.

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