



Radioprotective properties of nutraceutical Gonebazol: *In vivo* study

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ABSTRACT

BACKGROUND: *In vitro* investigation of radioprotective properties of novel nutraceutical Gonebazol (Biofarm, Belgrade) has displayed its remarkable potential to reduce chromosomal aberrations and micronuclei induced by γ rays. The goal of this study was to evaluate its protective properties *in vivo*. For this purpose, a group of medical staff performing invasive radiological diagnostics, that was identified to carry dicentric chromosomes in their lymphocytes, was selected for further monitoring. They consumed this nutraceutical for 3 weeks, 2 x 10 g per day (2 x 2 teaspoons dissolved in a glass of water).

METHODS: Hematological parameters, chromosomal aberrations and micronucleus frequency were examined at the beginning of monitoring, on day 10 and 21 of the treatment.

RESULTS: In the course of three weeks the incidence of chromosomal aberrations and micronuclei was significantly reduced (50%-80%), absolute number of granulocytes was lowered whereas the number of monocytes significantly was enhanced. This study has revealed that nutraceutical possesses immunomodulatory properties seen as an improving monocyte-macrophage activity, which inversely correlates with incidence of lymphocyte micronuclei ($r=0.75$, $p<0.05$).

CONCLUSION: Observed finding could be of particular importance in reducing cumulative effects of ionizing radiation in radiosensitive tissues and in preventing adverse health effects. No side effects were evidenced. Mechanisms of its immunomodulatory effects should be further examined.

KEY WORDS: Radiation, Ionizing; Occupational Exposure; Radiation-Protective Agents; Dietary Supplements; Chromosome Aberrations

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INTRODUCTION

The biological effects of ionizing radiation are divided into two categories: deterministic and stochastic effects. Deterministic effects have a threshold dose below which the biological response is not noticed. In fact, low doses of radiation - less than 50 mSv do not cause an immediate problem to any body organ, but spread out over long periods after exposure; the biological effects are at the DNA level and they may not be detected. The biological effects of low-dose radiation on living cells may result in three ways: 1) injured or damaged cells repair themselves, without residual damage, 2) cells die or 3) cells repair incorrectly themselves resulting in biological change.

Radiation-induced aberrations can be observed in human lymphocytes within few hours after the exposure; their frequency is related to the dose and quality of radiation and can be detected in the blood samples taken long after the exposure (1). Indirect estimation of chromosome damage can be obtained through the frequency of micronucleus, derived (mostly) from acentric fragments excluded from daughter nuclei at ana-telophase (2). The micronucleus frequency is used as alternative to the examination of lymphocyte aberrations (3), as predictive assay of radiosensitivity (4), and identification of cancer-prone individuals (5). Besides chromosomal aberrations, micronucleus test is frequently used in estimation

of somatic mutations due to occupational exposure to ionizing radiation. Our previous study has shown that 25% of medical staff performing catheterization carries dicentric chromosomes in their lymphocytes (6). Inverse correlation between incidence of micronuclei and apoptosis in persons carrying dicentric chromosomes was revealed, indicating that the chronic exposure to low doses of ionizing radiation disables the ability of leukocytes undergoing apoptosis, while the level of baseline micronucleus frequency correlates positively with the necrosis of leukocytes (7).

Exposition to low doses of ionizing radiation possibly influence the balance of the intracellular oxidant/antioxidant status, the level of ATP in cell, co-chaperones, the extension of induced membrane damage which lead cells to necrosis rather than apoptosis (8-12).

Individual variability in response to irradiation has been pointed out in numerous scientific publications (13-15) and explained as a variability in genes involved in DNA repair and biochemical determinants of resistance/ vulnerability to radiation (micronutrients and oxidative balance) (16).

Identification of radiosensitive and radio-resistant individuals opens the avenue to targeted pharmacologic and dietary chemopreventive interventions in radiosensitive subjects, particularly because some interventional procedures with long screening periods and multiple

image acquisition may give rise to deterministic effects in both staff and patients.

In vitro investigation of radioprotective properties of novel nutraceutical Gonebazol (Biofarm, Belgrade) has displayed its remarkable potential to reduce chromosomal aberrations and micronuclei induced by γ rays: incidence of micronuclei decreased for 69%, which was followed by intensified apoptosis of irradiated leukocytes (17).

The aim of this study is to evaluate radioprotective properties of this nutraceutical *in vivo*. For this purpose, a group of medical staff performing invasive radiological diagnostics, that was identified to carry dicentric in their lymphocytes, was selected for further monitoring. They voluntarily participated in this study and consumed nutraceutical for three weeks. Lymphocyte aberrations, micronuclei and hematological parameters were analyzed at the beginning of the monitoring, on day 10 and 21 of the treatment.

PATIENTS AND METHODS

Subjects

During routine health check-up of employees performing invasive diagnostic in cardiology, 10 out of 47 persons were selected according to the presence of dicentric chromosomes in their lymphocytes for further examination. Increased incidence of dicentrics and micronuclei in blood lymphocytes was followed by hematological disorders (mild leucopenia or leukocytosis and imbalance in ratio between lymphocytes, monocytes and granulocytes). The radiation doses measured by TLD dosimeters on the chest were under annual limits of 20 mSv. They were advised to continue with working activities in the zone of ionizing radiation and to consume nutraceuticals for 3 weeks, 2 x 10 g per day (2 x 2 teaspoons dissolved in a glass of water). Hematological parameters, micronuclei and chromosomal aberrations were checked on day 10 and 21 of the treatment.

Nutraceutical

Nutraceutical Gonebazol contains plant extracts (*Laminaria digitata* Khorbi, *Echinacea purpurea pulvis*) micronized clinoptilolite, pollen, propolis, vitamins B3, B6, A, E and C, Ca⁺² and Mg⁺², all preserved in honey.

Methods

Blood counts: Blood is taken in a test tube containing an anticoagulant (EDTA) to prevent clotting. The blood is well mixed (though not shaken) and paced on a special rack on the hematology analyzer CELL-DYN 3700: hemoglobin concentration, red blood cells (RBC), white blood cells (WBC), and counts of neutrophils, lymphocytes, monocytes, and eosinophils were obtained in numerical values and graphics.

Chromosome aberration analysis: A standard method, described in IAEA 2001, was used in evaluation of chromosomal aberrations: aliquots of heparinized whole blood (0.5 ml) were set in cultures containing PBmax-karyotyping medium (Invitrogen-Gibco). Cells were harvested for 48 hours after the initiation with presence of colchicine during the final 3 hours. The cultures were processed according to the standard protocol for chromosome aberration analysis. After staining 200 of them that were well spread and completed the first division metaphases per subject, were analyzed for unstable chromosome-type aberrations, i.e. dicentrics, centric rings, and excess acentrics. The scoring criteria were based on the IAEA recommendations (1).

Micronucleus test: For micronuclei analysis aliquots of heparinized whole blood (0.5 ml) were set in cultures containing PBmax-karyotyping medium (Invitrogen-Gibco). Cytochalasin B at a final concentration of 4 μ g/ml was added to the samples after 44 hours of incubation and the lymphocyte cultures were incubated for additional 24 hours. The micronuclei were prepared according to the method described by Fenech, M (18). Micronucleus slides were air-dried and stained in 2% alkaline Giemsa. At least 1000 binu-

cleated cells were scored per culture (using magnification x400 or x1000 when necessary) registering micronuclei according to the criteria of Countryman and Heddle (19).

Statistics

A statistical analysis of each of the targeted parameters was carried out using statistical software package Statistics, version 5.5 for MS Windows. The analysis considered the incidence of chromosomal aberrations, micronuclei in binucleated cells, number of leukocytes, absolute number of lymphocytes, granulocytes and monocytes. Descriptive statistics as well as the Spearman rank order correlation test was applied for the parameters under consideration. Correlation coefficients (R) were calculated at a significant level of p<0.05.

RESULTS

The results of hematological and cytogenetic analysis are listed in Tables 1-3. Individual data of leukocyte parameters and lymphocyte findings (Table 1) show the number of leukocytes, percentage and absolute number of lymphocytes, granulocytes and monocytes.

Table 1. Individual data of leukocyte parameters and lymphocyte findings (chromosomal aberrations and micronuclei) in medical staff performing invasive radiological methods

Subject number	Num of leukocytes	Lymphocytes %	Granulocytes %	Monocytes %	Incidence of breakage per cell	Incidence of micronuclei per cell			
1. *	5550	45	2497.5	44.1	2447.5	10.9	605	0.025	0.033
	5600	56	3138.2	36.5	2044	7.46	417.76	0.03	0.036
2. *	5400	56.8	3069.4	34.4	1857.6	8.76	473.04	0.025	0.028
	7800	41.1	3205.8	54.7	4266.6	4.2	327.6	0.035	0.039
	7100	35.9	2548.9	54.1	3841.1	10	710	0.015	0.039
3. *	5500	27.6	1518	61.2	3366	11.2	616	0	0.038
	5500	45	2475	44	2420	11	605	0.025	0.033
	5600	56	3138.2	36.5	2044	7.46	417.76	0.03	0.036
4. *	5400	56.8	3069.4	34.4	1857.6	8.76	473.04	0.025	0.028
	7820	41.2	3223.3	54.6	4269.7	4.2	327	0.035	0.039
	7100	35.9	2548.9	54.1	3841.1	10	710	0.015	0.039
5.	5500	27.6	1518	61.2	3366	11.2	616	0	0.038
	5300	37.4	1982.2	56	2968	6.6	349.8	0.015	0.024
	3900	31.2	1216.8	58.4	2277.6	10.4	405.6	0.03	0.023
6. *	4900	38.7	1898.3	52.2	2557.8	9.06	443.94	0.01	0.007
	4800	44.9	2155.2	50.9	2443.2	4.2	201.6	0.015	0.045
	5700	50.8	2895.6	43	2451	6.2	353.4	0.01	0.043
7. *	5600	48.2	2698.6	44.7	2503.2	7.11	398.16	0.005	0.018
	4200	51.1	2146.2	43.8	1839.6	5.1	214.2	0.015	0.038
	5300	52.6	2790.5	38.9	2061.7	8.45	447.85	0.035	0.041
8.	4600	60.2	2766.9	34	1564	5.85	269.1	0	0.011
	7900	40.5	3199.5	55.7	4400.3	3.8	300.2	0.01	0.016
	7700	39.5	3043	56	4312	4.48	345	0.015	0.017
9.	7700	47.4	3650.6	46.8	3603.6	5.79	445.83	0.005	0.034
	2800	47.2	1321.6	48.4	1355.2	4.4	123.2	0.015	0.025
	3400	43.3	1471.5	48.8	1659.2	7.92	269.28	0	0.027
10.	3100	47.4	1468.2	43.9	1360.9	8.74	270.94	0	0.011
	7100	36.4	2584.4	60.1	4267.1	3.5	248.5	0.025	0.023
	8900	24.6	2192.1	68.9	6132.1	6.47	575.83	0	0.023
	4600	40.4	1858.9	51.1	2350.6	8.49	390.54	0	0.021

* dicentric and ring chromosomes accompanied with acentric fragments were recorded

Considering chromosomal aberrations, the sum of exchange aberrations (dicentric or rings) and excess acentrics was expressed as an incidence of breakages per cell. Lymphocyte micronuclei were expressed as an incidence of micronuclei per binucleated cell. In six persons out of 10, incidence of chromosomal aberrations were higher than is annually allowed. Exchange type of chromosomal aberration were found (dicentric and ring chromosomes). Under this circumstances pause with work in the zone of ionizing radiation (at least three months) strongly is recommended.

Mean values of parameters under consideration are listed in Table 2, whereas correlation between analyzed biological endpoints is given in Table 3. Decreasing of granulocytes and slightly enhancement in number of monocytes was observed while using nutraceutical. Inverse relationship between number of monocytes and incidence of micronuclei was observed. Number of granulocytes correlates positively with number of monocytes (Table 3).

Table 2. Descriptive statistics of analyzed biological endpoints in entire group of examinees

	Begging of investigation				10 days of consuming Gonebazol				21 day of consuming Gonebazol			
	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD
Number of Leukocytes	5877.00	2800.00	7900.00	1734.73	6350.0	3400.00	8900.00	1540.74	5230.00	3100.00	7700.00	1148.96
Number of Lymphocytes	2479.07	1321.60	3223.30	615.97	2498.37	1216.80	3138.20	679.40	2351.63	1468.20	3650.6	790.86
Number of Granulocytes	3067.72	1355.20	4400.30	1142.19	3066.38	1659.20	6132.10	1423.27	2438.73	1360.90	3603.60	796.17
Number of Monocytes	330.21	123.20	605.00	160.50	465.24	269.28	710.00	151.21	456.86	269.10	616.00	130.93
Incidence of CA breakages per cell	0.022	0.01	0.035	0.009	0.018	0.0	0.035	0.013	0.007	0.0	0.025	0.010
Incidence of MN per BN cell	0.032	0.016	0.045	0.009	0.032	0.017	0.043	0.009	0.023	0.007	0.038	0.012

Table 3. Correlation coefficients (R) between analyzed biological endpoints at significance of $p < 0.05$ (Spearman rank order correlation test)

	CA 1	CA 2	CA 3	MN 3	Monocytes 2	Monocytes 3
Leukocytes	0.085				0.77	
Lymphocytes		-0.78				
Granulocytes	0.76				0.88	0.87
Monocytes				0.75		

CA - chromosomal aberrations
 MN - micronuclei
 1 - Beginning of the monitoring (day 0)
 2 - Control after 10 days.
 3 - Control after 21 days of consuming nutraceuticals

DISCUSSION

Although *in vitro* investigations are faster method for obtaining information of biological activity, *in vivo* effect hardly can be estimated using only *in vitro* investigation. Since this nutraceutical was examined in several authorized Institutions (Military Academy- Food Pharmacology Institute, Food and Drug Agency), and registered as a dietary supplement, occupationally overexposed radiation workers, voluntarily involved in this study, were advised to consume it and to administer 10 grams per day, divided in 2 equal doses dissolved in a glass of water. Ten persons participated in the study. Six of them carried dicentric and rings in their lymphocytes, which was followed by leukocyte formula disorders (mild granulocytosis followed by a mild lymphopenia). The incidence of micronuclei ranged from 23-45 per 1000 binucleated cells. Considering entire group, after 10 days of administration, the incidence of chromosomal aberrations was slightly higher, "long lived" chromosomal exchanges were found in some subjects (pericentric inversions, supernumerary dicentrics without accompanying acentric-chromosomal aberration). It is well known that radiation induced chromosomal aberrations persist in peripheral lymphocytes as long as lymphocyte lives, and because of that, they represent cells with genomic disbalance that can, under particular circumstances, lead the cell to oncogenesis. Incidence of micronuclei, in general, was at the same or slightly lower level. Number of leukocytes slightly increases, which is followed by the increase of lymphocytes and monocytes, whereas number of granulocytes remains almost the same.

After three weeks, chromosomal aberrations disappeared from peripheral blood lymphocytes in 9 out of 10 persons, whereas incidence of micronuclei decreased for 50-80 percent if compared with the status on the beginning of treatment. The number of leukocytes in the entire group of examinees was in physiological range. Absolute number of granulocytes was lowered, whereas increased number of monocytes, gained after 10 days of treatment, remained unchanged.

Adverse health effects of ionizing radiation are still very important field of investigation. The scientific community in the field of radiation protection agreed that cumulative effects of chronic exposure to ionizing radiation cannot be predicted neither as avoided (20-22). Therefore, the destabilization of the chromosomal structure through the DNA amplification or chromosomal rearrangements could lead to overexpression of oncogenes and cancer development. Chromosome aberrations in peripheral lymphocytes were established to be

an intermediate endpoint in carcinogenesis (23). Recently published studies (24-26) have also approved that the elevated frequency of micronuclei could also be related to an overall genetic instability, and predictive biomarkers of cancer prone individuals.

This study has shown that the nutraceutical Gonebazol significantly reduces the incidence of radiation-induced chromosome aberrations and micronuclei in persons occupationally overexposed to ionizing radiation. Its powerful radioprotective properties are partly obtained through scavenging capacities of vitamins. Biologically active compounds of *Laminaria digitata Khorbi*, particularly oligoglucan Laminarin (a linear beta-1,3 glucan) was identified as immunomodulator enhancing monocyte-macrophage activity (27). Homeostasis among leukocytes was obtained in short period (3 weeks). According to the results revealed in this study, nutraceuticals with such radioprotective properties strongly is recommended in radiation protection, particularly because some interventional procedures with long screening periods and multiple image acquisition may give rise to deterministic effects in both, staff and patients.

Precise mechanism of its radioprotective and immunomodulating activity should be examined further.

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