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IN VITRO AND IN VIVO STUDIES OF ANTIMUTAGENIC PROPERTIES OF BIOGINSENG IN MAMMALIAN CELLS

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In certain industries the level of mutagens still remains quite high. The possibility of accidents at chemical factories or atomic power stations, accompanied by massive discharge of mutagens into the environment, likewise cannot be ruled out. Hence the great urgency for a search for antimutagenic agents and, in particular, those possessing not only antimutagenic, but also other beneficial properties besides. Ginseng is used on quite a wide scale as an adaptogen, influencing biosynthetic, neurohumoral, and bioenergetic processes at all stages of formation of protection against stress and its aftereffects [1]. Emergency mobilization of plastic and energy-yielding resources of the cell under the influence of the panaxosides contained in ginseng has led to the suggestion that these plant glycosides probably have an antimutagenic action.

The aim of the investigation described below was to study the possibility of reducing the frequency of chromosomal aberrations arising under the influence of mutagens by preliminary addition of bioginseng to the culture medium for cells in vitro or its injection into an animal in vivo.

EXPERIMENTAL METHOD

To study the antimutagenic properties of ginseng we chose a preparation obtained from a culture of callus cells of ginseng, known as bioginseng. The technology of producing bioginseng has been worked out at the All-Union Research Institute of Biotechnology and has been introduced at a number of factories. The biomass of the ginseng tissue culture has been shown to be stable with respect to its biochemical parameters, and closely similar in composition to the native route

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TABLE 1. Effect of Bioginseng on Kinetics of Proliferation and Frequency of SCE in Chinese Hamster Cells

Preparation	Dose, μg/ml	Frequency of SCE per cell (x ± m)	Percentage of cells in mitoses			Proliferation index
			first	second	third	
Control		7,08±0,41	14,00	86,00	—	1,86
Bioginseng 1	10	5,60±0,34*	8,00	92,00	—	1,92
	100	6,76±0,61	4,44	91,12	4,44	2,00
	1000	—	100,00	—	—	1,00
Bioginseng 2	10	5,92±0,40*	11,21	87,85	0,93	1,89
	100	7,12±0,62	14,04	85,96	—	1,86
	1000	—	100,00	—	—	1,00

Legend. Compounds were added for the whole duration of culture (36 h). Bioginseng 1 obtained by freezing biomass of cells, bioginseng 2 obtained by alcoholic extraction. Asterisk indicates significant differences from control ($p < 0.05$).

[2]. The investigation was carried out on Chinese hamster cell cultures (clone 237) and on cells of Ehrlich's ascites carcinoma, grown in the mouse peritoneal cavity. Cells of clone 237 were grown in medium 199 with 10% inactivated bovine serum and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). To analyze sister chromatid exchanges (SCE) bioginseng was added to 24-h cell cultures in a concentration of 10-1000 μg/ml, bromodeoxyuridine (10 μg/ml) was added, and the sample was incubated at 37°C for 36 h. For analysis of chromosomal aberrations, a solution of bioginseng was added to the culture medium 24, 36, and 48 h after seeding of the cells, and the standard mutagen mitomycin C was added after 48 h, either directly to the culture medium or, after incubation with a solution of bioginseng for 10 min. Cells were incubated for 30 h. Fixation and staining of the preparations of metaphase chromosomes were carried out by the method in [3]. In one case nitrosomethylurea (100 mg/kg) was injected into mice with a tumor strain developing in their peritoneal cavity, and in the other case, a solution of bioginseng (30 mg/kg) was injected beforehand, and nitrosomethylurea was injected 2 h later. Preparations of metaphase chromosomes of ascites cells were obtained by the method in [4] and stained with Giemsa's stain in phosphate buffer (pH 6.8). Chromosomal aberrations were analyzed in 100 ascites cells for each mouse, and in 100 cells of the first mitosis for culture of clone 237. The frequency of SCE was analyzed in 25 cells of the second mitosis, with clear differential staining along the whole chromosome. The proliferation index was calculated after analysis of 100 cells with differential staining on the basis of the ratio between cells in the first and subsequent mitoses [5].

EXPERIMENTAL RESULTS

Investigations conducted on Chinese hamster cells showed that high doses of bioginseng (100 μg/ml) have a weak cytotoxic action on cells, expressed as inhibition of proliferation. For instance, by the 36th hour, the cells had managed to pass through only one replication cycle. Meanwhile lower concentrations of bioginseng, namely 100 and 10 μg/ml, had a stimulating effect on cell proliferation, and this action was most marked in the case of the preparation obtained by freezing the biomass (Table 1).

An increase in the frequency of SCE in the cells is a sensitive indicator of mutagenic action [6]. On addition of bioginseng to the culture medium in concentrations of 10 and 100 μg/ml, not an increase but a decrease in the frequency of SCE was noted, possible evidence of its antimutagenic action [7]. The protective properties of bioginseng under these experimental conditions were exhibited relative to spontaneous mutation.

The antimutagenic action of bioginseng also was observed relative to processes induced by chemical mutagens (Table 2). After addition of the mutagen mitomycin C in a concentration of 10^{-2} μg/ml to a culture of Chinese hamster cells, a marked increase was found in the number of induced chromosomal aberrations, namely up to 8% compared with 1.96% of aberrant cells in the control. Bioginseng preparations added 24 h or 5 min before the use of mitomycin C, or simultaneously with it, exhibited antimutagenic action and reduced the frequency of cells with chromosomal aberrations by

TABLE 2. Antimutagenic Action of Bioginseng against Chromosomal Aberrations Induced by Mitomycin C in Chinese Hamster Cells

Preparation	Dose, μg/ml	Time of administration of bioginseng	Percentage of cells with aberrations
Control			1,96
Bioginseng	10		1,90
Mitomycin C	0,01		8,00
Mitomycin C + bioginseng	0,01+10	24 h beforehand	4,21
		2 h beforehand	0,00
		5 min beforehand	3,85
Mixture of bioginseng and mitomycin C	10h 0,01		3,85

TABLE 3. Effect of Bioginseng on Chromosomal Aberrations Induced by Nitrosomethylurea (NMU) in Ehrlich's Ascites Tumor Cells in Mice

Preparation	Mean number of aberrations per cell	Percentage of cells with aberrations
NMU, 100 mg/kg	3,49	93
Bioginseng 1 + NMU	2,70	67
Bioginseng 2 + NMU	2,49	73

Legend. Bioginseng preparations injected 2 h before NMU.

half, In the case of administration of bioginseng 2 h before mitomycin C the mutagenic action of the antibiotic was exhibited virtually completely (Table 2). It must be pointed out that the spectrum of chromosomal aberrations induced by mitomycin C which we observed remained virtually unchanged after combined incubation with bioginseng (translocation, fragments, dicentrics), but the total number of these disturbances was reduced.

In experiments in vivo intraperitoneal injection of nitrosomethylurea into mice in a dose of 100 mg/kg caused the appearance of chromosomal aberrations in 93% of tumor cells. In those cases when mice received bioginseng before injection of the mutagen, the percentage of metaphases with aberrations fell to 67 (Table 3).

It will be clear from the results described above that bioginseng possesses an antimutagenic action, expressed as a substantial lowering of the level of chromosomal aberrations, both spontaneous and induced by chemical mutagens (nitrosomethylurea and mitomycin C). Comparison of these two bioginseng preparations showed that the one obtained by freezing a biomass of ginseng cells in culture has a more favorable action on the cells.

Chemical and biochemical analysis of the biomass of ginseng tissue culture shows that the content of extractives in it is 59% compared with 55% in the native route (the tissue is superior to the native route as regards protein content and total amino acids [8]). Analysis of the biological action of the ginseng preparation from tissue culture revealed physiological stimulation of cells of the guinea pig epidermis and also stimulation of protein and other types of metabolism in the skin [9].

The adaptogenic action of ginseng preparations is determined by its immunomodulating, gonadotropic, and antioxidant properties. The biological action of eleuterococcus, which exhibits a similar biological action, is known to influence the induction and transplantation of tumors, to inhibit metastasization, and to increase the efficacy of specific antitumor agents [11]. The antimutagenic action of eleuterococcus extracts also has been described [12, 13].

Because of the ability of ginseng extract and, correspondingly, of bioginseng, to exhibit antioxidative properties these preparations can exert a positive action on mutagenic compounds entering the body, and more specifically they can reduce mutagenic activity through assimilation of free radicals formed. These same properties may lie at the basis of the preventive action of ginseng against the common cold and other inflammatory diseases, when active release of endotoxins takes place in the body.

Nevertheless, the basis of the antimutagenic action of bioginseng may not be restricted to its antioxidative properties. The participation of other mechanisms and, in particular, stimulation of DNA repair processes, and synthesis of RNA-polymerases and other proteins, can be tentatively suggested.

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