GENETICS

Antimutagenic Activity of Lipidovit

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Antimutagenic properties of Lipidovit produced from the biomass of *Blakeslea trispora* fungi was studied by its effect on induction of chromosome aberrations by chemical mutagens dioxidine and cyclophosphamide in mouse bone marrow cells. Antimutagenic activity of Lipidovit depended on the scheme of treatment. It was maximum during pretreatment of animals (5 day) or administration in combination with mutagens (5 days). The preparation was ineffective after single administration in combination with mutagens. Lipidovit exhibited no comutagenic properties.

Key Words: *Lipidovit; antimutagen; chromosome aberrations; mice*

Various environmental factors produce mutations, which plays a major role in the development of hereditary diseases, congenial malformations, and malignant neoplasms [2,13].

The search and study of natural antimutagens allowed synthesizing new preparations that protect the human genome from negative effects of environmental genetic toxicants [5,12].

Experiments on mammals showed that vitamins, trace elements [5,11], β-carotene and other carotenoids [6,14], plant pigments [5,8,9], ubiquinones [3,5], and other compounds have antimutagenic activity [5,7]. It was important to evaluate the effect of combination treatment with antimutagens and natural complexes containing bioactive substances on induced mutagenesis [5,10]. Particular attention was given to the influence of Lipidovit (LV) on induced mutagenesis. This preparation is manufactured from *Blakeslea trispora* fungi containing ubiquinone, β-carotene, lycopene, and other bioactive substances [1,4].

Here we studied antimutagenic activity of LV in mice receiving chemical mutagens dioxidine (DN) and cyclophosphamide (CP) and compared antimutagenic properties of LV and β-carotene.

MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice aging 8-12 weeks and weighing 18-22 g (Stolbovaya nursery, Russian Academy of Medical Sciences). The animals were kept in a vivarium of the Institute of Pharmacology (Russian Academy of Medical Sciences) at the 12-h light/dark regimen and had free access to water and food.

DN (Farmakon, 200 mg/kg) or CP (Sigma, 20 mg/kg) was injected intraperitoneally to induce chromosome aberrations.

LV contained phospholipids (32.5±1.0%), monoglycerides (6.0±0.7%), sterols (5.0±0.9%), diglycerides (0.5±0.3%), free fatty acids (13.8±1.3%), triglycerides (30.0±2.5%), β-carotene (2.0±0.8%), lycopene (1.0±0.4%), total ubiquinones (5.0±0.2 mg/g), ergosterol (provitamin D_2, 3.2±0.3), E (200±10 mg/100 g), and K (0.4±0.1 mg/100 g).

LV was dissolved in olive oil and given perorally in doses of 5.4, 54.0, and 270.0 mg/kg (0.15, 1.50, and 7.50 mg/kg β-carotene, respectively). These doses were selected taking into account the data on antimutagenic

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activity of β-carotene [3,6]. There were three schemes of LV administration. In series I the mutagen and LV were administered once (acute experiment). In series II the mutagen was injected 1 time after 5-days pretreatment with LV. In series III the animals received LV in combination with the mutagen (combination treatment). The mice were killed 24 h after the last injection.

Cytogenetic assay determined chromosome aberrations in mouse bone marrow cells. A microscopic study was performed for single and paired fragments of chromosomes, exchanges, achromatic gaps, and cells with numerous chromosome aberrations (more than 5). The ratio of abnormal cells in control and treated animals was compared using φ-test. The experimental group consisted of 4-5 mice. We analyzed 100 metaphase plates from each animal.

RESULTS

DN induced chromosome aberrations in 9.5-10.3% cells (Table 1). Olive oil (control) did not modulate the cytostatic effect of DN.

LV in a single dose of 270 mg/kg decreased cytogenetic activity of DN by 38%. In lower doses this preparation had no effect on cytogenetic activity of the mutagen.

Pretreatment with LV produced a more potent antimutagenic effect in mice receiving DN. LV in various doses decreased cytogenetic activity of DN by 59-69%.

Antimutagenic activity of LV was also observed after 5-day combination treatment with the mutagen. Under these conditions, LV in various doses abolished the mutagenic effect of DN by 50% (Table 1).

These data show that LV possesses pronounced antimutagenic activity and decreased the cytogenetic effect of DN. The protective effect of LV was most pronounced after repeated treatment. In the acute experiment antimutagenic activity was observed only after administration of LV in the maximum dose.

CP markedly increased the count of cells with chromosome aberrations (Table 2). Administration of olive oil did not modulate cytogenetic activity of the mutagen in control mice.

In the acute experiment LV had no effect on cytogenetic activity of CP. However, pretreatment with LV

TABLE 1. Effect of LV on Cytogenetic Activity of DN in Mice

Experimental conditions	Cell count	Per 100 cells					Number of cells with chromosome aberrations (%, $M\pm m$)
		gaps	single fragments	paired fragments	exchanges	cells with multiple damages	
Control (intact animals)	500	1.2	1.4	0	0	0	2.4±0.7
Acute experiment							
DN, 200 mg/kg	400	0.5	6.0	0.3	0.8	3.8	10.3±1.5
+LV, mg/kg							
5.4	500	1.0	6.0	0	1.0	2.0	9.4±1.3
54	500	1.0	4.2	0	0.8	2.2	7.8±1.2
270	500	0.2	4.6	0.2	0.2	1.8	6.4±1.1*
Pretreatment							
DN, 200 mg/kg	400	1.0	7.3	0	0.5	3.0	10.5±1.5
+LV, mg/kg							
5.4	400	0.5	3.0	0	0.3	0.8	4.3±1.0*
54	400	0.5	2.0	0	0.8	0.8	3.3±0.9*
270	500	0.4	2.4	0.2	0.2	0.8	3.8±0.9*
Combined administration							
DN, 200 mg/kg	400	0	7.8	0.5	0.8	3.5	9.5±71.5
+LV, mg/kg							
5.4	400	0.5	2.3	0	0.3	1.5	4.5±1.2***
54	400	0.3	2.0	0	0.8	2.3	5.0±1.1**
270	500	0.6	2.8	0.2	0.4	1.0	4.4±0.9***

Note. Here and in Tables 2 and 3: *p<0.001, **p<0.05, and *p<0.01 compared to the control. "0", no statistically significant antimutagenic effect.

in various doses produced a potent antimutagenic effect and decreased cytogenetic activity of CP by 35-45%.

Mutagenic activity of CP decreased after combination treatment with LV. LV in doses of 54 and 270 mg/kg decreased the cytogenetic effect of CP by 44 and 38%, respectively (Table 2). It should be emphasized that LV in the lowest dose did not modulate cytogenetic activity of CP.

Our results indicate that LV has antimutagenic activity and decreases the cytogenetic effect of CP. The antimutagenic effect was most pronounced after pretreatment, but was absent in experiments with single administration of LV.

The data show that LV exhibits antimutagenic activity in relation to DN and CP. These mutagens differ in the mechanism of damage. DN is a direct mutagen inducing oxidative stress. CP is an indirect mutagen. The genotoxic mechanism of changes produced by CP includes alkylating and prooxidant effects [5]. The majority of environmental mutagens produce damage by these mechanisms. It can be hypothesized that LV produces a universal antimutagenic effect in relation to a variety of genetic toxicants.

We revealed two specific features of antimutagenic activity of LV. First, the protective effect of LV did not depend on its dose. And second, the antimutagenic effect of LV depended on the scheme of treatment. It should be emphasized that antimutagenic activity of LV was maximum after repeated administration. These features of antimutagenic activity are typical of most lipid-soluble antimutagens, including ubiquinones and carotenoids [3,5,6]. They are primarily related to kinetic and metabolic characteristics of these natural metabolites [3].

The protective antimutagenic effect of LV is probably related to the presence of carotenoids and ubiquinones. The content of these substances in LV is similar to the doses of β-carotene and ubiquinone producing the antimutagenic effect in relation to CP and DN [3,5,6]. For example, in our experiments the estimated doses of β-carotene were 0.15, 1.50, and 7.50 mg/kg. In previous experiments, this substance was administered in doses of 0.15, 1.50, and 15.00 mg/kg. It is important that LV and β-carotene were most effective after repeated treatment.

Published data show that LV is superior to β-carotene in antimutagenic activity (Table 3) [5,6]. The maximum protective effect of β-carotene did not exceed 50% and was observed in a lower number of animals. β-Carotene was inactive after pretreatment

TABLE 2. Effect of LV on Cytogenetic Activity of CP in Mice

Experimental conditions	Cell count	Per 100 cells					Number of cells with chromosome aberrations (%, *M*±*m*)
		gaps	single fragments	paired fragments	exchanges	cells with multiple damages	
Control (intact animals)	500	1.2	1.4	0	0	0	2.4±0.7
Acute experiment							
CP, 20 mg/kg	500	0.8	14.2	0.8	1.0	2.4	17.0±1.7
+LV, mg/kg							
5.4	500	1.0	13.6	0	0.6	1.4	13.0±1.5
54	400	0.5	19.0	0.5	2.3	2.5	19.3±2.0
270	500	0.8	12.6	0.2	1.6	0.6	13.4±1.5
Pretreatment							
CP, 20 mg/kg	400	1.5	11.8	0.3	1.8	0.5	12.3±1.6
+LV, mg/kg							
5.4	400	0.3	7.3	0.2	1.0	0	8.0±1.4**
54	500	0.8	7.0	0.2	0.2	0	7.4±1.2**
270	400	1.3	4.5	0	1.0	0.8	6.8±1.3***
Combined administration							
CP, 20 mg/kg	500	1.6	8.2	0.4	1.6	0	9.4±1.3
+LV, mg/kg							
5.4	500	0.6	6.8	0	1.0	0	7.4±1.2
54	500	0.6	4.8	0	1.6	0	5.4±1.0**
270	500	0.6	4.4	0.2	1.8	0.2	5.8±1.0**

TABLE 3. Comparison of Antimutagenic Activity of LV and β-Carotene

Doses	DN		CP	
	+LV	+β-carotene	+LV	+β-carotene
Acute experiment				
I	0*	0	0	0
II	0	0	0	0
III	38	0	0	0
Pretreatment				
I	59	0	35	0
II	69	42	40	0
III	64	57	45	0
Combined administration				
I	53	22	0	0
II	48	27	44	33
III	53	27	38	43

Note. Doses: I, 5.4 mg/kg LV and 0.15 mg/kg β-carotene; II, 54 mg/kg LV and 1.5 mg/kg β-carotene; III, 270 mg/kg LV and 15 mg/kg β-carotene. *Reduction of the effect, percents of a level observed after individual treatment with the mutagen.

with CP, while LV exhibited pronounced antimutagenic activity under these conditions. A greater antimutagenic activity of LV can be related to the presence of β-carotene, antimutagenic substances ubiquinone, lycopene, and vitamin E, and other unidentified antimutagens.

It should be emphasized that LV did not potentiate the effect of mutagens. The data indicate that LV possesses no undesirable comutagenic properties typical of various antimutagens and diminishing their protective effect on the genome [5,15].

LV blocks the cytogenetic effect of chemical toxicants with various mechanisms of action and possesses no comutagenic activity. Antimutagenic properties of LV should be studied in further experiments.

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