PHARMACOLOGY AND TOXICOLOGY

Effect of *Betula* Bark Extract on Spontaneous and Induced Mutagenesis in Mice

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Study was performed by counting cells with chromosomal aberrations in C57Bl/6 mice. Bark dry extract was given perorally in doses of 50, 150, 450, and 1500 mg/kg. Mutagens dioxidine and cyclophosphamide were injected intraperitoneally in doses of 200 and 20 mg/kg, respectively. Bark dry extract in doses of 150 and 1500 mg/kg did not possess cytogenetic activity. Bark dry extract in doses of 50, 150, and 450 mg/kg significantly decreased the cytogenetic effect of mutagens under various regimens of treatment with the preparation (single combined administration, 5-day pretreatment, and 5-day combined administration). Our results indicate that bark dry extract possesses antimutagenic properties.

Key Words: *bark dry extract; antimutagens; mice*

Birch bark (*Betula*) is a rich source of lupane triterpenoids betulin, lupeol, and betulinic acid. These compounds possess antiinflammatory, radioprotective, hepatoprotective, antiviral, antitumor, immunomodulatory, and antioxidant properties [2,4,6,9,12].

The substances possessing antioxidant and immunomodulatory properties often exhibit antimutagenic activity [3]. Antimutagenic activity was revealed in triterpenoids lupeol and betulinic acid [8,13].

The extract of birch bark (BETULA PENDULA Roth and BETULA PUBESCENS Ehrh) containing not less than 70% betulin is manufactured by the Berezovyi Mir Company. Bark dry extract (BDE) exhibits a variety of biological properties.

A genotoxicological study is required to determine antimutagenic activity of BDE in mammals (experiment) and evaluate the safety of this preparation (preclinical trial).

Here we studied the effects of BDE on spontaneous and dioxidine- or cyclophosphamide-induced mutagenesis in mice.

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MATERIALS AND METHODS

The method of counting chromosomal aberrations is extensively used to study mutagenic and antimutagenic properties of various chemical compounds [1,5].

Experiments were performed on male and female C57Bl/6 mice aging 8-12 weeks and weighing 22-25 g. The animals were kept in a vivarium of the Institute of Pharmacology under a 12:12-h light/dark regimen and standard conditions and had free access to water and food (balanced briquetted mixed fodder).

We used BDE (Berezovyi Mir) suspension in Tween 80 (Sigma). BDE was administered intragastrically in doses of 50, 150, 450, and 1500 mg/kg. Control animals received an equivalent volume of distilled water and detergent.

Mutagenesis was induced by a wide-spectrum antibacterial preparation dioxidine (DN, 1,4-di-N-oxide of 2,3-bis-(hydroxymethyl) quinoxaline, Farmakon) and cytostatic antitumor preparation cyclophosphamide (CP, N'-bis-(b-chloroethyl)-N'-O-trimethyl ester of phosphoric acid diamine, Deko Company) in doses of 200 and 20 mg/kg, respectively. Both mutagens were injected intraperitoneally.

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Mutagenic activity of BDE (150 and 1500 mg/kg perorally) in mice was studied according to methodical recommendations of the Pharmacological Committee (Russian Ministry of Health) [1].

Mutagen-modifying properties of BDE were determined in 3 series of experiments: single combined treatment with BDE and mutagen (acute experiment, series I); daily administration of BDE for 5 days, last treatment with BDE in combination with mutagen injection (pretreatment, series II); daily combined treatment with BDE and mutagen for 5 days (combined treatment, series III). The animals were killed 6 (series III) or 24 h after the last injection (series I and II).

Cytogenetic preparations of the femoral bone marrow were prepared by the standard air drying method [11]. Cytogenetic study determined the presence of cells with achromatic gaps, single and paired fragments of chromosomes, and various exchanges. A separate category (cells with multiple abnormalities) encompassed metaphases with more than 5 chromosomal aberrations. The ratio of cells with chromosomal aberrations in control and treated animals was compared by means of φ-test. The experimental groups consisted of 4-5 mice. We analyzed 100 metaphases from each animal.

RESULTS

Cytogenetic analysis of bone marrow cells showed that single and 5-day administration of 150 mg/kg BDE to male and female mice and single treatment of males with 1500 mg/kg BDE produced no mutagenic effects. The count of cells with chromosomal aberrations practically did not differ in treated and control animals.

Administration of BDE in a single dose of 200 mg/kg did not increase the count of cells with chromosomal aberrations (Table 1).

The number of aberrant metaphases in mice receiving 50 mg/kg BDE and DN was much lower compared to the positive control (53% decrease in the effect of DN). BDE in a dose of 150 mg/kg produce more pronounced antimutagenic effect. Further increase in the dose of BDE to 450 mg/kg was followed by a slight decrease in its antimutagenic activity (Table 1).

The antimutagenic effect of BDE was also revealed in experiments with CP (Table 1).

BDE in doses of 50 and 150 mg/kg had the same antimutagenic effect and decreased damaging activity of the mutagen by 60%. However, the antimutagenic effect was not observed after treatment with BDE in a higher dose (450 mg/kg). Under these conditions the count of aberrant metaphases did not differ from the positive control.

TABLE 1. Effect of Single Treatment with BDE on Mutagenesis Induced by DN and CP

Mutagen, BDE dose		Cell count	Number per 100 cells					Total number of abnormal metaphases, *M±m*, %	Decrease in mutagenic effect, %
			gaps	single fragments	paired fragments	exchanges	MCA		
Control		500	0.2	1.4	0	0	0	1.6±0.6	
DN, mg/kg		500	0	7.4	0.4	0.4	3.6	10.2±1.4	
DN and BDE	50 mg/kg	500	0	5.3	0	0	0.8	4.8±1.0**	53
	150 mg/kg	500	0.2	5.8	0.3	0	1.3	4.0±0.9*	61
	450 mg/kg	500	0	5.2	0	0	2.0	6.2±1.1***	33
CP, mg/kg		400	0.2	10.0	1.3	0.3	1.0	8.8±1.4	
CP and BDE	50 mg/kg	400	0.6	3.0	0.3	0	0	3.5±0.9**	60
	150 mg/kg	400	0.8	4.3	0	0	0	3.5±0.9**	60
	450 mg/kg	500	0.2	11.0	0.2	0	0	6.4±1.1	—

Note. MCA, cells with multiple chromosome abnormalities; —, not found. Here and in Tables 2 and 3: *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to 200 mg/kg DN and 20 mg/kg CP.

TABLE 2. Effect of 5-Day Pretreatment with BDE on Mutagenesis Induced by DN and CP

Mutagen, BDE dose		Cell count	Number per 100 cells					Total number of abnormal metaphases, $M \pm m$, %	Decrease in mutagenic effect, %
			gaps	single fragments	paired fragments	exchanges	MCA		
Control		500	0.2	1.4	0	0	0	1.6±0.6	
DN, mg/kg		500	0.2	11.4	0.4	0.6	4.4	9.8±1.3	
DN and BDE	50 mg/kg	400	0.4	5.3	0.3	0.3	2.0	5.0±1.1**	49
	150 mg/kg	500	0.2	5.8	0	0	0.4	3.6±0.8*	63
	450 mg/kg	500	0.2	4.6	0	0	0.2	2.6±0.7*	73
CP, mg/kg		500	0.6	15.0	0.6	1.0	1.6	12.0±1.5	
CP and BDE	50 mg/kg	400	0.4	6.2	0.2	0	0	5.5±1.1*	54
	150 mg/kg	500	0	6.0	0.2	0	0.2	4.2±0.9*	65
	450 mg/kg	500	0	7.2	0	0.2	0	4.4±0.9*	63

TABLE 3. Effect of 5-Day Combined Pretreatment with BDE on Mutagenesis Induced by DN and CP

Mutagen, BDE dose		Cell count	Number per 100 cells					Total number of abnormal metaphases, $M \pm m$, %	Decrease in mutagenic effect, %
			gaps	single fragments	paired fragments	exchanges	MCA		
Control		500	0.2	1.4	0	0	0	1.6±0.6	
DN, mg/kg		500	0.2	8.2	0.2	0	5.0	11.6±1.4	
DN and BDE	50 mg/kg	500	0.4	10.4	0	0.2	0.6	6.6±1.2**	43
	150 mg/kg	400	0.8	5.3	0.3	0	0.3	4.8±1.1*	59
	450 mg/kg	500	0.2	6.8	0.2	0	1.0	5.8±1.0*	50
CP, mg/kg		500	0.8	8.2	0.2	1.0	2.6	8.4±1.2	
CP and BDE	50 mg/kg	500	0.4	5.4	0	0	0	3.8±0.7**	54
	150 mg/kg	500	0.8	6.4	0.2	0	0	5.0±1.0***	40
	450 mg/kg	500	0.6	6.4	0	0.2	0.6	5.2±1.0***	38

BDE exhibited higher antimutagenic activity in series II (compared to single administration, Table 2). BDE in a dose of 50 mg/kg decreased the damaging effect of DN. The protective effect increased with an increasing the dose of BDE to 150 mg/kg. BDE in a dose of 450 mg/kg had maximum protective activity and completely blocked the damaging effect of DN. The count of aberrant metaphases in these mice did not differ from the level typical of spontaneous mutagenesis (control, Table 2).

After 5-day pretreatment antimutagenic activity of BDE surpassed that observed in experiments with single administration of the preparation and CP (Table 2). Study of aberrant metaphases showed that the cytogenetic effect of CP decreases by 54% in mice receiving 50 mg/kg BDE. The effect of BDE became more pronounced with increasing the dose of this preparation. As differentiated form single treatment, the antimutagenic effect was observed after administration of BDE in a dose of 450 mg/kg (Table 2).

Series III showed that the mutagenic effect of DN decreases by 43% after combined treatment with 50 mg/kg BDE. Pairwise comparison of the results revealed statistically significant differences between treated and positive control mice. The preparation in a dose of 150 mg/kg produced a more pronounced antimutagenic effect than 450 mg/kg BDE (Table 3).

BDE in various doses had an antimutagenic effect in CP-receiving mice. Study of aberrant metaphases showed that the mutagenic effect of CP decreases by 54% after combined treatment with 50 mg/kg BDE. BDE in doses of 150 and 450 mg/kg exhibited the same antimutagenic properties and decreased the damaging effect of CP (Table 3).

Our study indicates that BDE has antimutagenic activity. These results are consistent with published data on antimutagenic activity of betulinic acid and lupeol [8,13].

The antimutagenic effect was most pronounced after repeated pretreatment and administration of BDE in a dose of 150 mg/kg. Comparative study of antimutagenic activity showed that BDE is most effective in animals receiving DN.

Bark extracts, betulin (74% in the test sample), lupeol, and betulinic acid entering the composition of BDE possess high antioxidant activity [10]. BDE abolished the effect of prooxidant mutagen DN [3]. These data suggest that the antimutagenic effect of BDE is related to inhibition of free radical oxidation.

The cytogenetic effect of CP is mediated by lipid peroxidation products. Therefore, prevention of mutagen-produced changes can be related to antioxidant activity of BDE. It should be emphasized that CP acts as an indirect mitogen, whose effects are associated with the ability of its metabolites (*e.g.*, acrolein) to alkylate the DNA molecule [3,7]. The protective effect of DBE is probably realized via inhibition of cytochromes P450 playing a role in the metabolism of CP.

The antimutagenic effect of DBE can be mediated by various mechanisms. Previous studies showed that BDE stimulates production of interferons, which improves DNA reparation [3].

Published data [3] show that antimutagenic activity of natural compounds and their complexes does not exceed 50%. Apart from antimutagenic properties, they often have undesirable comutagenic activity. The advantage of BDE is that this preparation does not have comutagenic activity and produces a strong antimutagenic effect. The protective effect of single administration and pretreatment with BDE is not less than 60%.

Our results indicate that BDE has pronounced antimutagenic activity and produces no undesirable mutagenic and comutagenic effects in mammals.

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