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Antimutagenic Activity of Afobazole in Various Regimens of Treatment

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The influence of a new 2-mercaptobenzimidazole derivative afobazole on cytogenetic effects of dioxidine and cyclophosphamide was studied by counting chromosome aberrations in bone marrow cells of C57Bl/6 mice. Afobazole (1-100 mg/kg perorally) exhibited antimutagenic activity determined by its antioxidant properties. This activity depended on the dose and treatment shedule.

Key Words: *2-mercaptobenzimidazole; afobazole; chromosome aberrations; antimutagen; mice*

New genome-protecting drugs hold much promise for preventing consequences of induced mutagenesis. These drugs should have not only antimutagenic, but also other properties [1]. 2-Mercaptobenzimidazole (2-MBI) derivatives are of particular interest in this respect. Bemitil displaying antimutagenic, actoprotecting and immunomodulating properties is widely used in clinical practice for preventing mutagenic effects of dioxidine and genome protection in patients with systemic lupus erythematosus [1,2].

A new 2-MBI derivative afobazole (2-[2-(morpholino)ethylio]-5-ethoxybenzimidazole) synthesized at the Institute of Pharmacology acts as selective anxiolytic [4].

Here we studied antimutagenic properties of afobazole in mice.

MATERIALS AND METHODS

Experiments were performed on 8-12-week-old male C57Bl/6 mice kept in a vivarium (Institute of Pharmacology) at a 12-h light-dark cycle and free access to food and water.

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Broad-spectrum antibiotic dioxidine (DN, prooxidant mutagen [3]) was injected intraperitoneally in doses of 100 and 300 mg/kg. Cytostatic antineoplastic drug cyclophosphamide (CP, indirect alkylating mutagen) was injected intraperitoneally in a dose of 20 mg/kg [1]. Afobazole was dissolved in distilled water and administered perorally in doses of 1, 10, and 100 mg/kg (effective dose producing anxiolytic effect is 10 mg/kg).

In series I, the mutagen and afobazole were administered simultaneously 24 h before sacrifice. In series II, the mice received afobazole for 5 days and were injected with the mutagen 24 h before sacrifice (pretreatment). In series III, the animals were treated with the mutagen and afobazole for 5 days and killed 6 h after the last treatment (combined use).

Cytogenetic preparations of femoral bone marrow were prepared by the standard dry-air method [5]. Cells with chromosome gaps, single or paired chromosome fragments, and various exchanges were taken into account [6]. Metaphase with more than 5 chromosome aberrations were counted separately. The number of abnormal cells in the control and experimental groups were compared by φ test. In each series, 100 metaphase plates from 4-5 mice were analyzed.

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RESULTS

In series I, DN in doses of 100 and 300 mg/kg increased the number of aberrant metaphases compared to the control (Table 1), which is consistent with published data [1].

The number of aberrant cells in mice simultaneously treated with DN and afobazole was lower than after DN administration. The degree of induced mutagenesis did not differ from that of spontaneous mutagenesis (Table 1).

Afobazole in all doses markedly decreased the number of aberrant metaphases induced by 300 mg/kg DN (Table 1). Single administration of afobazole in doses of 1, 10, and 100 mg/kg abolished CP-induced changes.

In series II, repeated treatment with 1, 10, and 100 mg/kg afobazole for 5 days decreased the number of metaphases with chromosome aberrations induced by 100 and 300 mg/kg DN (Table 2).

Treatment with 1 mg/kg afobazole against the background of CP administration decreased the number of cells with chromosome aberrations (insignificantly); in doses of 10 and 100 mg/kg, afobazole markedly inhibited cytogenetic effects of CP (Table 2).

In series III, combined treatment with 1 and 10 mg/kg afobazole and 100 mg/kg DN had no effect on the number of aberrant metaphases. Afobazole in a dose of 100 mg/kg inhibited cytogenetic effects of DN (Table 3).

Afobazole in doses of 1 and 10 mg/kg had no effect on mutagenic activity of CP, while in a dose of 100 mg/kg this preparation decreased the number of aberrant metaphases (Table 3).

These results suggest that afobazole inhibits clastogenic effects of various mutagens (prooxidant DN and alkylating agent CP) to a different extent, which was probably related to various mechanisms of their effects. Afobazole was more potent in preventing mutagenic effects of DN than CP. Mutagenic activity of DN is associated with intensification of free radical production [3]. Therefore, the protective effect of afobazole is determined by its antioxidant properties [4]. It should be emphasized that afobazole is characterized by the absence of a dose-dependent shift from antimutagenic to co-mutagenic effect, which is typical of some antimutagens with antioxidant properties [1,3].

Antimutagenic activity of afobazole depends on the dose and administration shedule. This dependence is characteristic of antimutagens with antioxidant properties [1]. The protective effect of afobazole was less pronounced in the case of pretreatment or combined use with mutagens and manifested only at high doses of this drug. This can be explained by peculiarities of afobazole pharmacokinetics. Previous studies performed at the Laboratory of Pharmacokinetics (Institute of Pharmacology) showed that long-term administration of afobazole accelerates its elimination.

TABLE 1. Influence of Afobazole (A) on Clastogenic Effects of DN and CP in Male C57Bl/6 Mice (Acute Experiment)

Series		Cells	Per 100 cells					Aberrant metaphases, %	Inhibition of mutagenic effect, %
			gaps	fragments		exchanges	MA		
				single	paired				
Control		500	0.2	1.0	—	—	—	1.2±0.4	—
DN, 100 mg/kg		700	0.4	5.4	0.4	—	0.7	5.7±0.9	—
+A, mg/kg	1	700	0.4	3.4	—	—	—	2.3±0.6*	SM
	10	500	0.2	1.8	0.2	—	0.4	2.8±0.7***	SM
	100	500	0.2	2.0	—	—	0.4	2.0±0.6*	SM
DN, 300 mg/kg		500	0.4	19.0	1.6	0.4	9.4	22.8±1.8	—
+A, mg/kg	1	400	0.4	7.2	0.4	0.2	4.0	12.8±1.7*	44
	10	500	0.6	7.0	0.4	—	0.8	5.6±1.7*	75
	100	500	0.4	9.8	0.8	—	1.8	10.4±1.4*	54
CP, 20 mg/kg		400	1.0	11.0	1.0	1.0	3.0	15.3±1.7	—
+A, mg/kg	1	500	0.8	8.2	0.6	0.2	2.0	10.2±1.6***	33
	10	500	0.4	2.6	0.6	—	1.6	4.6±0.9*	69
	100	400	0.8	4.7	—	—	0.3	5.5±1.1*	64

Note. Here and in Tables 2 and 3: *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to the control. SM: inhibition of mutagenic effects to the level of spontaneous mutagenesis; MA: multiple aberrations.

TABLE 2. Influence of Afobazole (A) on Clastogenic Effects of DN and CP in Male C57Bl/6 Mice (Pretreatment)

Series		Cells	Per 100 cells					Aberrant metaphases, %	Inhibition of mutagenic effect, %
			gaps	fragments		exchanges	MA		
				single	paired				
Control		500	0.2	1.0	—	—	—	1.2±0.4	—
DN, 100 mg/kg		500	0.4	4.0	0.6	—	0.2	5.0±1.0	—
+A, mg/kg	1	500	0.6	1.4	—	—	—	1.4±0.5*	SM
	10	500	0.2	1.4	—	—	—	1.4±0.5*	SM
	100	500	0.5	1.6	—	—	—	1.8±0.6*	SM
DN, 300 mg/kg		500	—	15.4	1.0	1.6	6.6	17.6±1.7	—
+A, mg/kg	1	500	—	13.4	0.6	0.2	5.0	10.6±1.4**	40
	10	500	—	5.6	0.4	—	1.6	5.2±1.0*	71
	100	400	0.3	7.8	0.3	0.3	1.0	5.0±1.0*	72
CP, 20 mg/kg		400	0.5	18.3	2.3	1.2	3.3	19.3±2.0	—
+A, mg/kg	1	500	0.2	19.8	1.2	0.4	0.6	15.4±1.6	0
	10	500	0.4	16.0	0.2	0.2	0.2	12.2±1.5***	37
	100	500	0.2	16.8	—	—	—	10.6±1.4*	45

TABLE 3. Influence of Afobazole (A) on Clastogenic Effects of DN and CP in Male C57Bl/6 Mice (Combined Use)

Series		Cells	Per 100 cells					Aberrant metaphases, %	Inhibition of mutagenic effect, %
			gaps	fragments		exchanges	MA		
				single	paired				
Control		500	0.2	1.0	—	—	—	1.2±0.4	—
DN, 100 mg/kg		500	0.2	11.0	1.0	0.2	0.2	10.6±1.4	—
+A, mg/kg	1	500	0.6	9.8	0.6	—	—	9.2±1.3	0
	10	500	0.6	8.2	0.4	—	—	8.4±1.2	0
	100	500	0.4	5.0	—	—	—	4.8±0.9*	55
CP, 20 mg/kg		500	0.4	20.6	2.2	1.0	2.8	22.2±1.8	—
+A, mg/kg	1	500	0.6	18.4	1.8	—	0.8	16.2±1.6	0
	10	500	0.6	18.2	0.8	—	1.2	16.2±1.6	0
	100	500	0.4	14.0	0.8	—	—	12.4±1.5*	44

Antimutagenic effect of afobazole is comparable to that of other 2-MBI derivatives. Afobazole is superior to bemitil (single treatment) [1,3] and produces the protective effect in much lower concentrations (repeated treatment).

Thus, afobazole possesses not only antioxidant, but also antimutagenic activity, which depends on the dose and administration shedule. Therefore, afobazole holds much promise for protecting human genome from mutagens.

REFERENCES

1. A. D. Durnev and S. B. Seredenin, *Mutagens: Screening and Pharmacological Prevention* [in Russian], Moscow (1998).
2. T. A. Lisitsina, A. D. Durnev, M. M. Ivanova, *et al.*, *Eksp. Klin. Farmakol.*, **62**, No. 5, 38-41 (1999).
3. S. B. Seredenin and A. D. Durnev, *Pharmacological Protection of the Genome* [in Russian], Moscow (1992).
4. S. B. Seredenin, T. A. Voronina, G. G. Neznamov, *et al.*, *Vestn. Ros. Akad. Med. Nauk*, No. 1, 3-9 (1998).
5. G. R. Mohn and J. Ellenberger, *Mutat. Res.*, **32**, 331-360 (1976).
6. R. S. Preston, B. J. Dean, *et al.*, *Ibid.*, **189**, 157-165 (1987).