genetic control and is inherited as a dominant trait. The gene (or genes) is linked with the H-2 complex, and according to the preliminary data, is located on the left of the S region.

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MUTAGENIC AND ANTIMUTAGENIC PROPERTIES OF BEMITIL

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Proof of the role of induced mutations in the spread of hereditary diseases and in the development of malignant neoplasms has stimulated research aimed at the discovery of chemical mutagens and the search for compounds with modifying, antimutagenic properties [1, 2]. Such compounds can be used in conjunction with drugs which have a genotoxic effect, but which have not been withdrawn from practice because of their medical importance [7]. It is evident that the search for modifiers should take place among substances capable not only of preventing or reducing harmful effects, but at the same time, reinforcing the therapeutic effect. Such compounds include certain psychotropic agents, tranquilizers, and actoprotectors, widely used in combination pharmacotherapy.

The aim of this investigation was to study the effect of a new actoprotector, bemitil, a derivative of 2-mercaptobenzimidazole, on the level of spontaneous mutations and of mutations induced by alkylating agents.

EXPERIMENTAL METHOD

The investigation was conducted in accordance with the technical recommendations of the Ministry of Health of the USSR on mutagenicity testing [4]. The action of bemitil in a concentration of 10 mg/ml on the spontaneous mutation level in *Drosophila melanogaster* was studied

TABLE 1. Effect of Bemitil on Level of Recessive Sex-Linked Lethal Mutation in *D. melanogaster* (M ± m)

Experimental conditions	Total number of chromosomes investigated	Frequency of mutations, %
Control	753	0,4±0,2
Bemitil (10 mg/ml)	685	0,4±0,2

Legend. Here and in Tables 2-4: *P > 0.05 compared with control.

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TABLE 2. Effect of Bemitil in a Dose of 180 mg/kg on DLM in C57BL/6 Mice (M ± m)

Experimental conditions	Stage of spermatogenesis	Number of effective matings, %	Total number of embryos		Postimplantation mortality, %
			living	dead	
Control	Mature spermatozoa	58,4	255	12	4,5±1,3
Bemitil		71,6	329	15	4,4±1,1*
Control	Late spermatids	70,0	333	21	5,9±1,2
Bemitil		58,4	256	22	7,9±1,6*
Control	Early and medium spermatids	65,0	304	20	6,2±1,3
Bemitil		58,3	240	18	7,0±1,6*
Control	Late spermatocytes	65,0	282	15	5,1±1,3
Bemitil		66,6	315	19	5,7±1,3*

TABLE 3. Effect of Bemitil on Level of Chromosomal Injuries in Bone Marrow Cells of C57BL/6 Mice (M ± m)

Experimental conditions	Dose of bemitil, mg/kg	Number of		Gaps	Single fragments	Paired fragments	Number of chromosomal aberrations	Number of injured metaphases, %
		mice	cells	per 100 cells				
Control	—	5	500	0,6	0,6	—	0,6	1,2±0,5
Bemitil given 24 h beforehand	1,8	5	500	0,4	0,6	0,2	0,8	1,2±0,5*
	18	5	500	0,6	0,6	0,4	1,0	1,6±0,6*
Daily for 7 days	1,8	5	500	0,6	0,6	—	0,6	1,2±0,5*

TABLE 4. Effect of Bemitil on Level of Chromosomal Aberrations in Culture of Human Peripheral Blood Lymphocytes (M ± m)

Experimental conditions	Concentration of drug, μg/ml	Number of metaphases studied	Number of aberrant metaphases, %
Control	—	578	1,2±0,4
Bemitil	0,018	197	2,5±1,1*
	0,18	100	1,0±0,9*
	1,8	137	2,2±1,2*

TABLE 5. Modifying Action of Bemitil on Mutagenic Effects of Fotrin in a Dose of 7 mg/kg (M ± m)

Experimental conditions	Number of		Gaps	Single fragments	Paired fragments	Exchanges	Number of chromosomal aberrations	Number of cells with multiple lesions	Total number of injured cells
	mice	cells	per 100 cells						
Control	22	2150	0,7	0,6	0,05	—	0,65	—	1,4±0,3
Fotrin	20	2000	1,8	13,7	2,1	0,3	16,6	0,1	15,8±0,8[a]
Fotrin + bemitil (0,018 mg/kg)	5	500	1,8	12,2	2,4	—	14,6	—	15,0±1,6[a]
Fotrin + bemitil (0,18 mg/kg)	5	376	2,9	11,4	2,1	—	13,5	—	15,6±1,9[a]
Fotrin + bemitil (1,8 mg/kg)	10	985	1,1	7,5	0,1	0,5	7,5	—	8,0±0,9[a,b]
Fotrin + bemitil (18 mg/kg)	10	829	1,4	6,8	0,2	0,4	7,4	0,12	8,3±0,9[a,b]

Legend. a) P < 0.001 compared with control, b) the same, compared with Fotrin.

TABLE 6. Modifying Action of Bemitil on Mutagenic Effects of Fopurin in a Dose of 24 mg/kg (M ± m)

Experimental conditions	Number of		Gaps	Single fragments	Paired fragments	Exchanges	Number of chromosomal aberrations	Number of injured metaphases, %
	mice	cells	per 100 cells					
Control	22	2150	0,7	0,6	0,05	—	0 65	1,4±0,3
Fopurin	10	1000	2,4	11,5	0,5	0,1	12,1	13,1±1,1[a]
Fopurin + bemitil (0,18 mg/kg)	5	500	1,0	8,4	0,4	—	8,8	9,6±1,3[a,b]
Fopurin + bemitil (1,8 mg/kg	5	500	1,4	5,8	0.4	—	6,2	7,2±1,2[a,b]
Fopurin + bemitil (1,8 mg/kg)	5	400	1,0	4,8	1,0	—	5,8	6,5±1,2[a,b]

Legend. *a*) P < 0.001 compared with control, b) the same, compared with Fopurin, c) P < 0.05 compared with Fopurin.

by the Meller-5 method. Changes in mammalian germ cells were assessed by the method of counting dominant lethal mutations (DLM) [3]. Bemitil was injected intraperitoneally in a single dose of 180 mg/kg into male CBA × C57BL/6 mice weighing 20-22 g (from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR).

The effect of bemitil on the spontaneous and chemically induced level of cytogenetic injuries in bone marrow cells of C57BL/6 mice [4] was studied after intraperitoneal injection of the drug in doses of 1.8 and 18 mg/kg separately, and of 0.18-18 mg/kg together with the alkylating agents Fotrin (7 mg/kg) and Fopurin (24 mg/kg). The action of bemitil also was studied when given daily for 7 days in a dose of 1.8 mg/kg.

Cytogenetic effects *in vitro* were studied in short-term culture of human peripheral blood lymphocytes, when bemitil was added to the culture in final concentrations of 0.018, 0.18, and 1.8 μg/ml, simultaneously with phytohemagglutinin (PHA). The duration of exposure was 72 h.

EXPERIMENTAL RESULTS

Analysis of the effect of bemitil on the level of recessive sex-linked lethal mutations in *D. melanogaster* showed the absence of mutagenic effects (Table 1).

In a dose of 180 mg/kg bemitil caused no increase in DLM at pre- and postmeiotic states of spermatogenesis, so that its harmful action on germ cells could be ruled out (Table 2).

Investigation of the cytogenetic activity of bemitil *in vitro* and *in vivo* likewise revealed no increase in the number of chromosomal injuries in the mouse bone marrow cells or human peripheral blood lymphocytes (Tables 3 and 4).

On the basis of all the results of these experiments it can thus be concluded that bemitil has no mutagenic activity over a wide range of doses. This conclusion is an essential condition for the use of this new drug in practice.

The results of the study of the effect of bemitil on the level of chromosomal injuries induced in mouse bone marrow cells by Fotrin and Fopurin are given in Tables 5 and 6.

Fotrin (7 mg/kg) increased the yield of abnormal cells from 1.5 to 15.8%. The spectrum of the lesions produced included 1.8 gaps, 13.7 single fragments, 2.1 paired fragments, and 0.3 aberration of exchange type for every 100 metaphase plates, in agreement with data in the literature [5]. In doses of 0.018 and 0.18 mg/kg bemitil did not modify the effects of Fotrin. However, if the dose of bemitil was increased to 1.8 mg/kg, corresponding to ED_{50} for C57BL/6 mice, a reduction of the total number of induced mutations by 50% was discovered. The modifying action of bemitil was found not to depend on dose (Table 5).

Administration of Fopurin, as a standard mutagen, in a dose of 24 mg/kg caused an increase in the number of injured metaphases to 13.1%. These data confirm the results of previous investigations [6]. Together with Fopurin, bemitil in doses of 0.18 to 18 mg/kg, led to a reduction of the above parameter to 50%, with a decrease in the frequency of all types of chromosomal aberrations. By contrast with the results of the previous experiments, the modifying effect was dose-dependent in character. It must therefore be concluded that there are

differences in the biochemical processes that determine the protective properties of bemitil against the effects of Fotrin and Fopurin, but in order to understand the pathways of their realization, an additional investigation of the common stages of the molecular mechanisms of formation of the effects of these compounds, interaction on which prevents the formation of mutations, is necessary.

It can be concluded from the results of these experiments that bemitil is able to reduce the genotoxic effect of alkylating agents and it can accordingly be recommended for use as a protector for combined administration with drugs possessing mutagenic activity.

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