ability for biotransformation. It is characteristic that in the case of intravenous injection, I and II are not registered in the blood. Evidently in this method of administration, the metabolite II is not formed, since there is no effect of "first passage of the drug through the liver."

LITERATURE CITED

1. A. L. Ékonomov, A. P. Rodionov, V. P. Zherdev, et al., Xenobiotica, 9, 503-510 (1979).
2. S. B. Seredenin, V. G. Zin'kovskii, N. Ya. Golovenko, et al., Khim.-farm. Zh., No. 9, 23-26 (1981).
3. N. Ya. Golovenko, V. G. Zin'kovskii, A. V. Bogatskii, et al., Khim.-farm. Zh., No. 1, 21-26 (1979).
4. T. A. Voronina, "Pharmacology of benzdiazepine compounds," Doctoral Dissertation [in Russian], Moscow (1978).
5. A. L. Ékonomov and V. P. Zherdev, Khim.-farm. Zh., No. 8, 97-100 (1980).
6. V. N. Solov'ev, A. A. Firsov, and V. A. Filov, Farmakokinetika, Moscow (1980).
7. J. G. Wagner, Fundamentals of Clinical Pharmacokinetics, Hamilton (1975).
8. M. Gibaldi and D. Perrier, Pharmacokinetics, New York (1975).
9. M. Rowland, J. Pharm. Sci., 61, 70 (1972).
10. J. C. K. Loo and S. Riegelman, J. Pharm. Sci., 57, 918-928 (1968).
11. R. A. Ronfeld and L. Z. Benet, J. Pharm. Sci., 66, 178-180 (1977).
12. H. Boxenbaum, J. Pharmacokinet. Biopharm., 8, 165-176 (1980).

INFLUENCE OF THE COMPLEX OF CATECHINS FROM TEA LEAVES ON THE APPEARANCE OF GENETIC AND CHEMOTHERAPEUTIC PROPERTIES OF THE ANTIBACTERIAL DRUG DIOXIDINE

G. N. Zolotareva, I. V. Berezovskaya, M. N. Zaprometov, Yu. A. Revazova, É. A. Rudzit, I. N. Faddeeva, É. A. Akaeva, V. A. Bratslavskii, A. F. Girich, A. S. Kinzirskii, G. R. Koverova, D. A. Kulikova, O. P. Chernikova, and T. P. Radkevich

UDC 615.281:547.863.1].065:616-055.5/.7].015.25.615.322:[547.978.4:582.823

A number of valuable drugs possess mutagenic properties. In view of this, the question arises of the possibility of "neutralization" of the mutagenicity of drugs while preserving their therapeutic properties.

We first demonstrated the reality of the solution of this problem with the use of an antimutagen. It was shown on a number of objects that hexamidine, which possesses antimutagenic activity with respect to spontaneous mutation [1], lowers the level of mutation induced by the valuable antibacterial drug dioxidine [2]. At the same time, it was shown in experiment *in vitro* and on infected animals that hexamidine did not affect the therapeutic effectiveness of dioxidine, as well as its tolerability, including its acute toxicity and the changes in the activity of the central nervous system induced by dioxidine [2].

In the plan of expanding investigations on the prophylaxis of the mutagenicity of valuable drugs using antimutagens, we turned our attention to some natural phenolic compounds of plant origin. Earlier we showed that (—)-epicatechin and (—)-epigallocatechin, isolated from tea leaves, lower the level of spontaneous mutation in plant cells [3]. These polyphenols are the most important components of green tea, providing for its taste and toning qualities; they possess P-vitamin [4] and antioxidant activity [5].

The purpose of the present work was to study the ability of the complex of catechins isolated from the tea plant and containing the above-mentioned catechins to affect spontaneous mutation in mouse and *Drosophila* bone marrow cells, and also to exhibit antimutagenic

Scientific-Research Institute for the Biological Testing of Chemical Compounds, Moscow Province.

Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 17, No. 2, pp. 138-142, February, 1983. Original article submitted March 2, 1982.

0091-150X/83/1702-0082$07.50

TABLE 1. Influence of the Complex of Catechins on the Cytogenetic Activity of Dioxidine in Mouse Bone Marrow Cells

Variant of experiment	Metaphases			Metaphases with multiple chromosome aberrations*	
	total number	aberrant			
		abs.	%±m	abs.	% of number of aberrant metaphases
Complex of catechins, 1 day	246	2	0,81±0,57	0	0
Complex of catechins, 6 days	332	3	0,90±0,51	0	0
Complex of catechins, 11 days	283	1	0,35±0,35	0	0
Dioxidine, 270 mg/kg, 1 day	460	89	19,35±1,84	42	47,19
Complex of catechins, 1 day + dioxidine	443	47	10,61±1,46†	12	25,53
Complex of catechins, 6 days + dioxidine	495	35	7,07±1,15‡	10	28,57
Complex of catechins, 11 days + dioxidine	390	34	8,72±1,43‡	11	32,35
Control	319	2	0,63±0,44	0	0

*Cells with multiple chromosome aberrations are included in the column of total number of metaphases.
†P < 0.01.
‡P < 0.001.

properties with respect to mutation induced by dioxidine on the same test objects.

A parallel evaluation was made of the influence of the phenolic complex on the bacterial action of dioxidine in experiments *in vitro* and *in vivo* as well as on its acute toxicity.

MATERIALS AND METHODS

Dioxidine is an original domestic preparation, highly effective in suppurative infections, including those with a severe course, which cannot be treated with other antibacterial agents [6]. Dioxidine exhibits substantial mutagenic activity, including both gene and chromosomal mutations on a number of test objects [7].

The complex of catechins isolated from tea leaves was used as a potential antimutagen. The main components of this complex are: (−)-epigallocatechin gallate, (−)-epigallocatechin, (−)-epicatechin gallate (listed in order of decreasing relative fraction) [8].

The genetic aetion of the complex of catechins and a combination of it with dioxidine was estimated according to the induction of chromosome aberrations in mouse bone marrow cells, and also by consideration of the recessive sex-linked mutations in *Drosophila*. In an evaluation of chromosome aberrations in bone marrow metaphases of first-generation CBA × C57Bl/6 hybrid mice, two months of age, our modification of the method of Ford and Hammerton [9] was used. The catechin complex was administered in a single intraperitoneal injection in a dose of 100 mg/kg 12, 24, and 48 h before sacrifice. In a study of the effects of the catechin complex on induced mutation, three series of investigations were conducted: the catechin complex in a dose of 10 mg/kg was injected once intraperitoneally, as well as daily for 6 and 11 days. Dioxidine in a dose of 270 mg/kg was injected intraperitoneally 24 h after the last injection of the catechin complex. The animals were sacrificed after 24 h. In each variant five animals were used. Statistical treatment of the results was performed considering the Student criterion [10].

The frequency of appearance of recessive sex-linked lethal mutations in *Drosophila* was verified according to the standard Meller-5 procedure. Males of the D-32 line and intact females of the Meller-5 line [Sn (1) Sc^{SIL} $Sc^{82}Ln$ (1)S, $Sc^{8}Sc^{55}W^{a}B$ (BASc)] were used.

The drug was administered with food, exposure was for 72 h. Dioxidine and the catechin complex were used in a concentration of 5 mg/ml in a 5% sugar solution, each. Statistical treatment was performed with the aid of the precise Fisher formula and the χ^2 criterion.

Colibacillary sepsis was induced with a 24 h agar culture of *E. coli* 675. The microbial suspension was prepared in physiological solution, mixed with a 0.25% agar solution in a 1:4 ratio, and administered intraperitoneally to noninbred mice in a dose of 100 million microbial cells in a volume of 1 ml. Dioxidine in doses of 50 mg/kg or less, as well as the

TABLE 2. Influence of the Complex of Catechins on the Level of Recessive Lethal Mutations Induced by Dioxidine in *Drosophila*

Variant of experiment	Total number of cultures examined in F_2	Including those with recessive lethal mutations		Statistical index χ^2
		abs.	% ± m	
Dioxidine, 5 mg/kg	1808	17	0,94±0,23	18,76
Complex of catechins, 5 mg/kg	1358	2	0,15±0,10	0,004
Dioxidine + complex of catechins	1009	3	0,29±0,17	0,19
Control	3559	6	0,16±0,06	

complex of catechins in a dose of 10 mg/kg, were injected subcutaneously in physiological solution. The animals were treated once immediately after infection. The results of the experiments were determined according to the survival of the animals on the 10th day of the observations. The average therapeutic doses (TD_{50}) were calculated by the method of [11]. The model of suppurative burn infection was described earlier [12]. Dioxidine in a dose of 100 mg/kg was injected subcutaneously once a day in physiological solution; the complex of catechins in doses of 10 and 5 mg/kg was injected subcutaneously in the same solvent. The time of treatment was seven days. The therapeutic effect was judged according to the survival of the animals.

The acute toxicity of dioxidine and its combinations with the complex of catechins was studied on noninbred sexually mature male mice 2.5 months of age. Dioxidine was injected intraperitoneally in the form of an aqueous suspension with Tween-80. In the study dioxidine was simultaneously injected intraperitoneally and introduced intragastrically in the complex of catechins in a dose of 20 mg/kg. The state of the experimental animals and their death were recorded for two weeks. The average monthly dose was calculated by the method of [13].

RESULTS OF THE INVESTIGATIONS

Influence of the Catechin Complex on Spontaneous and Dioxidine-Induced Mutation in Mouse Bone Marrow Cells. The complex of catechins did not change the level of natural mutation in mouse cells in the case of a single injection in a dose of 100 mg/kg. Thus, when the mice were treated for 12, 24, and 48 h the percentage of mutant cells in the experimental variants was 0.71 ± 0.40, 0.49 ± 0.34, and 0.48 ± 0.33; in the control the values were 0.40 ± 0.39, 0.42 ± 0.42, and 0, respectively. In the case of daily administration of the catechin complex in a dose of 10 mg/kg for 1, 6, and 11 days, the level of mutation also corresponded to the control (Table 1). Chromosome aberrations in the experimental and control variants are represented by single deletions.

Dioxidine in a dose of 270 mg/kg induced chromosome aberrations in the cells, which corresponds to the data described earlier [14]. The complex of catechins substantially lowered the level of mutation induced by dioxidine, in the case of their combined used (see Table 1). This effect was manifested both in the case of a single administration of the drug to the animals at 24 h and in the case of daily administration for several days, preceding the administration of dioxidine. In the later case the effect was more substantial.

The genetic action of dioxidine in mouse bone marrow cells is usually accompanied by the appearance of a substantial number of cells with multiple aberrations [14]. The number of such cells in the case of combined use of dioxidine and a complex of catechins was lowered by 30-40%, depending on the variant (see Table 1). Thus, the decrease in the number of mutant cells induced by dioxidine in the bone marrow of mice was accompanied by a change in the qualitative characteristics of the mutation process under consideration — a decrease in the number of cells with multiple aberrations against a background of a decrease in the level of mutation induced by dioxidine. We recorded this effect earlier in the case of combined use of dioxidine with hexamidine [2]. The observed analogy indicates a similarity of the processes lying at the basis of the antimutagenic action of these chemically different drugs.

Influence of the Complex of Cathechins on Spontaneous and Dioxidine-Induced Mutation in *Drosophila*. In the test of assay of recessive sex-linked lethal mutations in *Drosophila*, dioxidine gave a mutagenic effect. In turn, the complex of catechins had no mutagenic effect. In the case of combined use of the drugs, the level of mutations induced by dioxidine

TABLE 3. Chemotherapeutic Activity of Dioxidine in the Case of Separate Use and Joint Use with the Catechin Complex on a Model of Colibacillary Sepsis in Mice

Drug	Daily dose, mg/kg	Survival	
		abs.	%
Dioxidine	50	10/10	100
	25	9/10	90
	12,5	0,10	0
	6,25	0/10	0
	TD_{50}	20	
Dioxidine + catechin complex in dose 10 mg/kg	50	10/10	100
	25	8/10	80
	12,5	0/10	0
	6,25	0/10	0
	TD_{50}	20	
Control		0/20	0

Note. Here and in Table 4 the number of surviving animals is in the numerator, the number of animals in the group in the denominator.

TABLE 4. Chemotherapeutic Effectiveness of Dioxidine in Separate Use and in Joint Use with the Catechin Complex in Suppurative Burn Infection in Mice

Variant of experiment	Survival	
	abs.	%
Control 1, burn	8/10	80
Control 2, burn + infection	0/10	0
Treatment with dioxidine in a dose of 100 mg/kg	7/10	70
Treatment with dioxidine + catechin complex in dose 10 mg/kg	15/20	75
Treatment with dioxidine + catechin complex in dose 5 mg/kg	6/10	60

was lowered to the control indices ($P > 0.05$). In this case the differences between the frequencies of mutations induced by the combination of drugs and by dioxidine alone were statistically significant ($P < 0.05$) (Table 2).

Influence of the Catechin Complex on the Chemotherapeutic Activity of Dioxidine. In experiments *in vitro* in concentrations of 100 and 10 μg/ml, the preparation did not affect the bacteriostatic activity of dioxidine with respect to *Salmonella typhi*, *E. coli*, and *Bacillus pyocyaneus*; in a concentration of 1000 μg/ml the catechin complex itself exhibited a bacteriostatic effect with respect to these strains of microorganisms. As can be seen from Table 3, in colibacillary sepsis in mice, dioxidine in doses of 50-25 mg/kg had a high chemotherapeutic effect (100-90% of the animals survived). When the dose was lowered to 6.25 mg/kg, the drug did not give a therapeutic effect. In the case of combined use of dioxidine and the cathechin complex, similar results were obtained. The average therapeutic dose for dioxidine was 20 mg/kg.

From Table 4 it follows that in the control group of animals with a burn without infection, 20% of the mice die within seven days. In the group of animals with a burn and infection with *Bacillus pyocyaneus*, 100% of the mice died during the experiment. After treatment with dioxidine, 70% of the animals survived. In the case of combined treatment with dioxidine and the catechin complex, the survival of the animals was the same as in the case of isolated use of dioxidine, i.e., the complex in doses of 10 and 5 mg/kg had practically no effect on the therapeutic effectiveness of dioxidine.

Influence of the Catechin Complex on the Acute Toxicity of Dioxidine in Mice. As a result of the experiments it was established that LD_{50} of dioxidine in the case of intraperitoneal injection of the drug was 750 mg/kg (662-840 mg/kg). LD_{50} of dioxidine and a combination of it with the catechin complex in a dose of 20 mg/kg is equal to 780 mg/kg (696-873 mg/kg); the differences in acute toxicity of dioxidine and its combination with the catechin complex are statistically insignificant ($PR < fpr$).

Our investigation shows the promise of the search for preparations for the prophylaxis of the mutagenicity of valuable drugs among biogenic compounds that had previously exhibited antimutagenic activity with respect to spontaneous mutation.

LITERATURE CITED

1. G. N. Zolotareva, É. A. Akaeva, and R. I. Goncharova, Dokl. Akad. Nauk SSSR, 246, No. 2, 469-471 (1979).
2. G. N. Zolotareva, L. M. Fonshtein, É. A. Rudzit, et al., Khim.-farm. Zh., No. 7, 30-35 (1980).

3. G. N. Vinkler, M. N. Zaprometov, and V. K. Shcherbakov, Dokl. Akad. Nauk SSSR, 177, No. 3, 699-702 (1967).
4. A. L. Kursanov, M. N. Zaprometov, and N. N. Erofeeva, Biokhimiya, No. 6, 729-733 (1952).
5. N. A. Zakharova, G. N. Bogdanov, and M. N. Zaprometov et al., Zh. Obshch. Khim., 42, No. 6, 1414-1420 (1972).
6. E. N. Padeiskaya, G. N. Pershin, B. M. Kostyuchenko, et al., Khim.-farm. Zh., No. 8, 139-146 (1977).
7. L. M. Fonshtein, G. N. Zolotareva, Yu. A. Revazova, et al., Genetika, No. 5, 900-908 (1978).
8. M. N. Zaprometov, Catechin Biochemistry [in Russian], Moscow (1964), pp. 244-262.
9. G. N. Zolotareva, É. A. Akaeva, É. N. Iskhakova, et al., Khim.-farm. Zh. No. 3, 19-22 (1978).
10. P. F. Rokitskii, Introduction to Statistical Genetics [in Russian], Minsk (1978).
11. L. J. Reed and H. Muench, Am. J. Hyg., 27, 493-497 (1938).
12. A. F. Girich, V. P. Yakovlev, and É. A. Rudzit, Farmakol. Toksikol., No. 1, 90-93 (1976).
13. J. T. Litchfield and F. J. Wilcoxon, J. Pharmacol. Exp. Ther., 96, 99-113 (1949).
14. G. N. Zolotareva, É. N. Meksina, and É. A. Akaeva, Khim.-farm. Zh., No. 11, 16-18, (1978).