MOLECULAR-BIOLOGICAL PROBLEMS IN THE CREATION OF DRUGS AND STUDY OF THE MECHANISM OF THEIR ACTION

EFFECTS OF PRODIGIOSAN ON THE CYTOGENETIC AND CHEMOTHERAPEUTIC PROPERTIES OF DIOXIDINE

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Some effective chemotherapeutic preparations for the treatment of bacterial and protozoan infections—nitrofurans, nalidixic acid, certain sulfanilamides, isoniazid, as well as compounds from the class of quinoxaline di-N-oxides, i.e., carbadox, dioxidine, and quinoxidine—have a mutagenic effect [1-6].

Preparations that exhibit mutagenic activity should in principle be removed from circulation. And yet, the problem of preserving valuable drugs for clinical practice should be recognized as extremely urgent.

One of the ways to solve this problem is to seek antimutagens that balance out the mutagenic activity of drugs under the conditions of the living organism. That this approach is realistic is confirmed by our earlier findings in a study of the antimutagenic activity of the anticonvulsive preparation hexamidine. Hexamidine lowered the level of mutation induced by dioxidine on various biological specimens including animals, without changing the chemotherapeutic effect of dioxidine and without exhibiting any side effects [7].

This work presents the results of a study of the lipopolysaccharide prodigiosan as a potential inhibitor of the mutagenic action of dioxidine on mouse bone marrow cells.

Dioxidine is a highly effective domestic drug, used for the treatment of urogenital and burn infections, including those caused by *Bacillus pyocyaneus* [8].

Prodigiosan is a domestic biogenic preparation, obtained from the bacterium *Prodigiosum* [9]. It has a nonspecific stimulating effect: It activates the pituitary–adrenal cortex system and stimulates the phagocytic activity of the reticuloendothelial system [10]. Moreover, prodigiosan stimulates the resorption of tumors in animals and also prevents the formation of tumors and has a radioprotective effect [11].

The influence of prodigiosan on the nature of spontaneous mutation, as well as on the manifestation of the genetic effect of dioxidine, was studied according to the frequency of induction of chromosome aberrations in bone marrow cells of 2-month-old male hybrid mice CBA × C57B1/6 and DBA × C57B1/6. A modified method of Ford and Hamerton was used [12]. From Tables 1 and 2 it is evident that prodigiosan in doses of 400 and 40 μg/kg does not change the level of spontaneous mutation of bone marrow cells of mice of different strains, both after a single subcutaneous injection 12, 24, or 48 h before sacrifice of the animals and for a period of three days with three injections of the preparation.

Dioxidine in doses of 140 and 270 μg/kg, injected intraperitoneally, causes the appearance of mutant cells in the bone marrow, including cells with multiple aberrations (10 or more in each cell). Subcutaneous injection of prodigiosan in a dose 40 μg/kg eliminates the mutagenic action of dioxidine. The effect depends on the system of administration of prodigiosan, as well as on the dose of dioxidine administered. The action of prodigiosan was most effective when a smaller dose of dioxidine, close to the therapeutic (for mice), was used.

Daily injections of prodigiosan over a period of three days, as well as 12, 24, and 48 h before the administration of dioxidine, were equally effective: The number of chromosome aberrations was lowered to the control indices.

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TABLE 1. Effects of Prodigiosan (40 μg/kg) on the Appearance on the Mutagenic Effect of Dioxidine in Bone Marrow Cells of DBA × C57Bl/6 Mice

Substance	Dose of dioxidine, mg/kg	Time of action, h	Scheme of administration of prodigiosan	Total number of metaphases	Aberrant metaphases		Number of metaphases with multiple chromosome aberrations
					abs.	% ± m	
Control	—	—	—	345	3	0,87±0,49	—
P	—	12	—	750	3	0,85±0,49	—
P	—	24	—	305	1	0,32±0,32	—
P	—	48	—	400	1	0,25±0,02	—
P	—	72	—	386	2	0,51±0,36	—
P	—	—	3-day administration	317	2	0,63±0,44	—
D	270	—	—	400	58	14,50±1,76	33
D	140	—	—	309	28	9,06±1,63	14
P + D	270	—	Simultaneously	358	34	9,49±1,54	24
P + D	270	—	12 h before D	315	19	6,03±1,34	2
P + D	270	—	24 h before D	320	20	6,17±1,33	—
P + D	270	—	48 h before D	306	44	14,37±2,00	34
P + D	270	—	2 days P, third day P + D simultaneously	320	16	5,00±1,21	9
P + D	140	—	12 h before D	322	9	2,79±0,91	2
P + D	140	—	24 h before D	324	8	2,46±0,86	2
P + D	140	—	2 days P, third day P + D simultaneously	331	6	1,81±0,73	—

Note. P) Prodigiosan; D) dioxidine.

TABLE 2. Effects of Prodigiosan (400 μg/kg) on the Level of Spontaneous Mutation in Bone Marrow Cells of CBA × C57Bl/6 Mice

Time of action of prodigiosan, h	Number of metaphases studied	Aberrant metaphase	
		abs.	% ± m
12	300	0	0
24	595	5	0,84±0,37
48	622	6	0,96±0,39
Control (total)	457	4	0,87±0,43

When dioxidine was used in the maximum dose, the level of its cytogenetic action was most significantly lowered in the case of a single injection of prodigiosan 12 or 24 h before administration of dioxidine, and also in the case of daily administration of prodigiosan for three days. Administration of prodigiosan 48 h before the injection of dioxidine did not eliminate the mutagenic action of the latter, just as in the case of a simultaneous injection of both preparations at 24 h.

When prodigiosan was administered simultaneously with dioxidine, it effectively prevented the appearance of cells with multiple aberrations or significantly decreased their number. A similar effect of reduction of the number of cells with multiple aberrations against a background of a general decrease in the level of mutation induced by dioxidine was recorded earlier in the case of combined use of dioxidine with the antimutagen hexamidine [7]. The observed analogy is an indication of the similarity of the processes lying at the basis of the antimutagenic action of such chemically different preparations as hexamidine and prodigiosan.

TABLE 3. Chemotherapeutic Effectiveness of Dioxidine and Prodigiosan in Colibacillary Infection in Mice

Preparation	Daily dose of dioxidine, mg/kg	Number of animals	Survival
Dioxidine	50	6	6/6
	25	6	6/6
	12,5	6	3/6
	6,25	6	2/6
Dioxidine + prodigiosan 400 μg/kg	50	6	5/6
	25	6	6/6
	12,5	6	6/6
	6,25	6	4/6
Control	—	9	2/9
Prodigiosan	—	6	0/6

Note. In the numerator: number of surviving animals; in the denominator: number of animals in the group.

The chemotherapeutic effect of dioxidine was increased when it was administered jointly with prodigiosan: Injection of prodigiosan 24 h before infection with *E. coli* 675 and treatment with dioxidine halved the minimum effective dose of dioxidine (from 25 to 12.5 mg/kg). Isolated administration of prodigiosan to infected mice somewhat lengthened their lifetimes (Table 3).

Thus, the bacterial lipopolysaccharide prodigiosan, while not affecting the spontaneous mutation level in mouse bone marrow cells, reduces the mutagenic action of dioxidine. Moreover, the total number of mutant cells is decreased, including the number of cells with multiple aberrations. The therapeutic action of dioxidine on mice is preserved and even enhanced when it is combined with prodigiosan.

It is known that prodigiosan, introduced into the organism, stimulates endogenous interferon formation [9]. Possibly the antimutagenic effect of prodigiosan observed under the experimental conditions is associated with its induction of interferon, which, as has been shown earlier [13, 14], possesses antimutagenic properties.

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