EFFECT OF BENZ(A)ANTHRACENE AS AN INDUCER OF MULTIPURPOSE OXIDASES ON THE TOXIC AND ANTITUMOR ACTION OF N,N-DI-[2-CHLOROETHYL]ANILINE

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The toxicity of the alkylating antitumor compound N,N-di-[2-chloroethyl]aniline can be considerably reduced by preliminary administration of benz(a)anthracene, an inducer of multipurpose oxidases, to rats. In this way animals with tumors can tolerate larger doses of the compound with a correspondingly enhanced therapeutic effect.

The high toxicity of existing antitumor preparations is a handicap to their use in the chemotherapy of malignant neoplasms. This accounts for the great interest in the study of cell systems whose activation would enable the tolerance of normal tissues to the toxic action of these compounds to be increased.

Research into the mechanism of resistance of tumor cells to the action of chemical carcinogens has shown that in the cells of most tumors the system of inducing enzymes, known as multipurpose oxidases, is disturbed [1, 3, 4, 7]. The enzymes of this system play an important role in the detoxication of foreign compounds entering the cell [2, 8]. In the cells of normal tissues activity of the multipurpose oxidases can be increased severalfold by treatment with various inducers, of which the most active are polycyclic hydrocarbons and barbiturates [6, 8, 9]. In the cells of most tumors so far studied this enzyme system is virtually uninducible [6, 8].

Advantage can be taken of these facts in order to obtain a selective increase in the resistance of normal tissues against the toxic action of antitumor compounds metabolized by this enzyme system.

The object of the investigation described below was to discover whether the tolerance of rats to the toxic action of one of the chloroethylamines can be increased by means of an inducer of the multipurpose oxidase system and to study how this is reflected in the antitumor activity of the compound.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats. Benz(a)anthracene [9] was chosen as the compound increasing the activity of the multipurpose oxidases, and N,N-di-[2-chloroethyl]aniline, known in the Soviet literature as "lymphochin" [5], was chosen as the toxic and antitumor compound. Multipurpose oxidases metabolize a wide variety of compounds which have only one common property, solubility in lipids [7, 8]. Evidently lymphochin itself, with a high affinity of lipids, is metabolized by this enzyme system, and benz(a)anthracene (BA) must stimulate its metabolic inactivation.

In the experiments of group 1 the effect of BA on the toxic action of lymphochin was investigated. BA in a dose of 20 mg in 2.5 ml sunflower oil was injected into the stomach, and this was followed after 48 h by a further single injection of various doses of oily solution of lymphochin in a concentration of 30 mg/ml, also into the stomach. Instead of BA the control animals received the corresponding volume of sunflower oil. The animals were kept under observation for at least 30 days after administration of lymphochin.

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TABLE 1. Toxicity of Lymphochin for Rats Receiving and Not Receiving Benz(a)anthracene

Dose of lymphochin (in mg/kg)	Ratio bet. no. of dying rats and no. in exper.			P
	—	oil	BA	
100	0/14	—	—	—
150	9/11	3/5	—	—
200	12/13	5/5	0/15	<0,05
250	14/15	9/12	1/24	<0,05
300	—	—	13/16	—

TABLE 2. Action of Lymphochin on Sarcoma M-1 in Rats Receiving and Not Receiving Benz(a)anthracene

Dose of lymphochin (in mg/kg)	BA	Number of rats		
		total	dying from toxicosis	cured
—	—	16	0	0
—	+	12	0	0
100	—	22	4	3
200	—	9	8	1
100	+	9	0	0
200	+	23	2	12

Legend: (+) compound given; (−) compound not given.

In the experiments of group 2 the effect of BA on the antitumor activity of lymphochin was studied. In these experiments a sarcoma M-1 was grafted subcutaneously on the left side of the animal's trunk. Animals with a tumor measuring not less than 3 cm^2 were taken for the experiment 12-14 days after grafting. BA and lymphochin were given just as in the experiments of group 1. The dynamics of growth and regression of the tumors was studied by determining their volume from three measurements every 6-8 days throughout the experiment. The tumor was regarded as cured if it did not recur during 20-40 days from the time of its complete disappearance. Statistical analysis of the results was carried out by the chi-square method.

EXPERIMENTAL RESULTS

Preliminary treatment of the rats with BA sharply reduces the toxic action of lymphochin on these animals. As Table 1 shows, rats receiving no additional treatment tolerated a single dose of 100 mg/kg lymphochin given into the stomach, and most animals died after a dose of 150 mg/kg. Preliminary administration of sunflower oil had no significant effect on the toxic action of lymphochin.

Treatment of the animals with BA 48 h before administration of lymphochin made it possible for the dose of the compound to be increased by 2.5 times, up to 250 mg/kg, without harm. Death of the animals receiving BA occurred after administration of lymphochin in a dose of about 300 mg/kg.

The lymphochin had a stronger toxic action on animals with sarcoma M-1 than on normal animals, but in the latter BA considerably reduced the toxicity of the compound (Table 2). This meant that the dose of lymphochin available for experimental treatment could be increased to something of the order of 200 mg/kg. Under those conditions, with minimal toxic action a marked antitumor effect was obtained, and one which could not have been achieved by the use of equitoxic doses of lymphochin without the inducer (Table 2; Fig. 1). It will be clear from Table 2 that administration of lymphochin to rats in a dose of 100 mg/kg without induction resulted in cure of three of 22 animals; meanwhile a dose of 200 mg/kg, which is lethal under ordinary conditions, when combined with BA resulted in complete absorption of the tumors in 12 of 23 animals (difference significant: $P < 0.01$), with the same level of toxicity.

In rats receiving preliminary BA treatment lymphochin in a dose of 100 mg/kg exhibited neither toxic nor antitumor effect. These animals differed very little in appearance and behavior from the controls, and tumors grew in them just as effectively (Fig. 1). Administration of BA without lymphochin to the rats had no effect on the rate of growth of the tumors.

The results thus showed that preliminary treatment of rats with the polycylic hydrocarbon BA significantly increases their tolerance to the toxic action of lymphochin. As an antitumor agent lymphochin possesses low selectivity relative to sarcoma M-1. Even if toxic doses of the compound (100 mg/kg) are used its antitumor effect is minimal. It can be increased by increasing the dose, but at the same time the toxicity is increased. A vicious circle arises, such as is characteristic of chemotherapeutic compounds with a low margin of therapeutic safety.

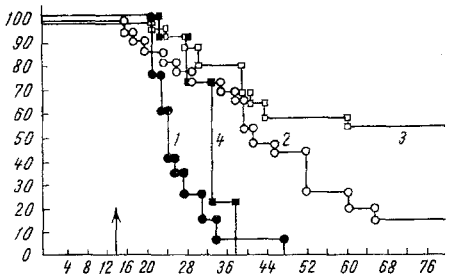

Fig. 1. Dynamics of death of rats during treatment of sarcoma M-1 with lymphochin. Ordinate, number of rats surviving (in percent of initial number); abscissa, time from grafting sarcoma (in days). 1) Control; 2) lymphochin (100 mg/kg); 3) BA plus lymphochin (200 mg/kg); 4) BA plus lymphoching (100 mg/kg). Arrow denotes injection of lymphochin.

Deficiency of multipurpose oxidases in tumor cells, as expressed by a low level of inducibility, can be utilized in the search for a way out of this situation. By means of an inducer (in this case BA) the metabolic inactivation of the chemotherapeutic compound in normal cells can be increased, and their tolerance to the compound can thereby be raised. The detoxicating power of tumor cells under these circumstances undergoes little change or remains at its previous level, and for this reason the margin between the dose toxic to the animal and the dose toxic to the tumor is widened.

The essential factor is that the liver cells, which are exposed to the action of the inducer, must metabolize the compound more actively and reduce its concentration in the body as a whole. As a result, as Fig. 1 and Table 2 show, with ordinary doses of lymphochin (100 mg/kg) its body level in rats treated with BA falls below the therapeutic level.

By the use of maximally high doses of lymphochin (200 mg/kg) it was possible to obtain a higher concentration of the compound in rats receiving BA which did not cause death of the animals but which was sufficient to suppress growth of the tumors in 50% of them.

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