EFFECT OF RHYTHM OF ADMINISTRATION OF 1,2-DIMETHYLHYDRAZINE ON ITS CARCINOGENIC ACTIVITY

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The rhythm of injection of 1,2-dimethylhydrazine (DMH), in the same total dose, affects the ultimate carcinogenic effect. The most widespread development of intestinal tumors is observed if DMH is given once a week in a dose of 21 mg/kg body weight. Daily injection in a dose of 3 mg/kg leads to less marked tumor development, limited to the large intestine. However, under these conditions pronounced degenerative and necrobiotic, and in a few cases neoplastic changes develop in the liver. The frequent administration of small doses of the carcinogen leads to a marked decrease in the cytochrome P_{450} content in the microsomes of the liver; this evidently delays the metabolism of DMH, its conversion into its end product, and binding of the latter with macromolecules of the enterocytes.

KEY WORDS: metabolism of 1,2-dimethylhydrazine; intestinal tumors; rhythm of administration of carcinogen; cytochromes P_{450} and b_5.

The carcinogenic effect is known to depend [5] on the total dose of the substance given and on the lifespan of the experimental animals. However, various modifying factors are also very important in this respect. One such factor is the rhythm of carcinogenic action. In other regions of pathology the role of the rhythm of action of pathogenic factors on the development of the pathological process has been clearly demonstrated [4]. A change in the frequency of development of intestinal tumors and in their location has been found following intrarectal injection of the highly reactive carcinogen N-methylnitrosourea into mice under different conditions [7].

The object of this investigation was to study the carcinogenic effect of 1,2-dimethylhydrazine (DMH), which has a resorptive method of action, depending on the rhythm of its administration to rats receiving the same total dose. In addition, changes in DMH metabolism associated with differences in the character of its action and capable of shedding light on differences in the carcinogenic effect under these conditions also were investigated. Attention was concentrated on interaction between the carcinogen and the nucleic acids and proteins of various organs, for a leading role is ascribed to this effect in carcinogenesis [2]. Considering that cytochromes P_{450} and b_5 are key enzymes in the metabolism of carcinogens [6], their content in the microsomes of the liver also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 90 noninbred male albino rats from the Rappolovo nursery, Academy of Medical Sciences of the USSR, weighing 200-250 g, on 60 of which chronic experiments were performed for the induction of intestinal tumors. There were three series of experiments (20 rats in each series). DMH was injected subcutaneously in accordance with the following schemes: 1) daily in a dose of 3 mg/kg body weight; 2) weekly in a dose of 21 mg/kg, and 3) once every 2 weeks in a dose of 42 mg/kg; in all cases the total dose was the same. The animals were killed at the same times after the beginning of DMH administration and underwent macroscopic and microscopic investigation. The remaining 30 rats were divided into three equal groups: animals of group 1 received DMH in a dose of 3 mg/kg daily, those of group 2 received 21 mg/kg weekly. In these two groups the carcinogen was administered for 2 weeks and the total dose was the same, namely 42 mg/

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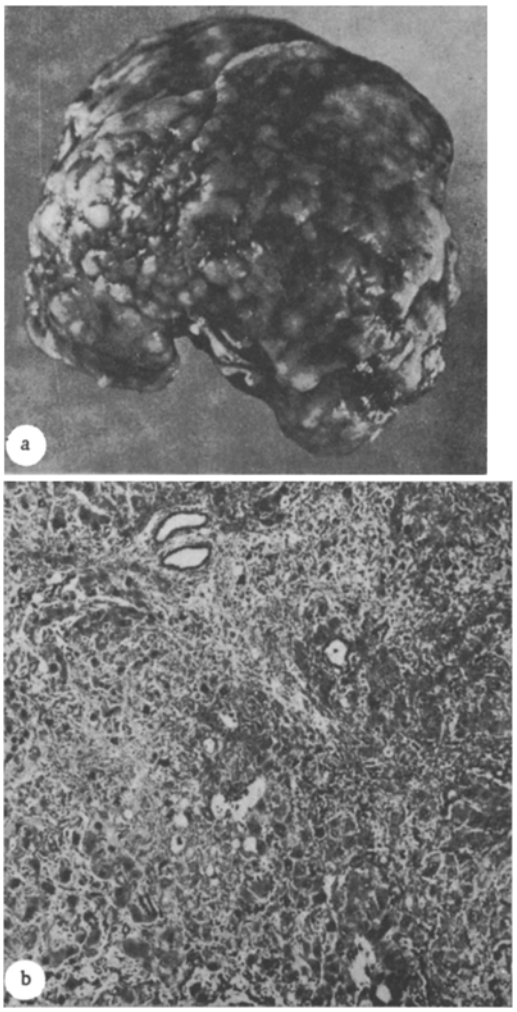

Fig. 1. Morphological changes in liver after injection of DMH in a dose of 3 mg/kg daily: a) macroscopic appearance; nodular liver as in cirrhosis (2×); b) extensive areas of necrosis and loss of normal structure of hepatic trabeculae (hematoxylin-eosin, 80×).

kg. The animals of the third (control) group received no special preliminary treatment. On the 15th day after the beginning of the experiments half of the animals of all groups received a subcutaneous injection of DMH-^{3}H (specific activity 12 mCi/g) in a dose of 21 mg/kg. The rats were killed 9 h after injection of the labeled carcinogen, i.e., at the peak of methylation of the macromolecules [1, 2]. The methods of injection of the carcinogen and of isolation and determination of radioactivity of the nucleic acids and proteins were described previously [1, 2].

The content of cytochromes P_{450} and b_5 was determined by the method of Omura and Sato [8] in the remaining animals of all three groups.

EXPERIMENTAL RESULTS

The experiments in which DMH was given in different sessional doses but the same total dose showed that the carcinogenic effect in these cases depends on the rhythm of entry of the carcinogen into the animal's body (Table 1).

As Table 1 shows, in the animals receiving DMH in a dose of 21 mg/kg weekly multiple tumors usually developed simultaneously in several segments of the intestine. Meanwhile, after daily injection of small doses

TABLE 1. Dependence of Carcinogenic Action of DMH on Rhythm of Its Administration

Rhythm of injection of DMH and sessional dose	Mean total dose of carcinogen, mg/kg	Mean quantity of DMH injected	Mean duration of experiment, days	Number of animals surviving until appearance of first tumor	Number of rats with different degrees of involvement of regions of intestine*																			
					duodenum				small intestine				cecum				large intestine				rectum			
					I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Daily, 3 mg/kg	528±11,2	178	178±3,9	12†	—	—	—	—	—	—	—	—	—	—	—	—	4	3	3	--	—	—	—	—
Weekly, 21 mg/kg	514±10,8	24	170±3,6	13	1	—	5	1	1	2	2	1	1	1	3	1	—	1	4	8	2	2	—	—
Once every 2 weeks, 42 mg/kg	524±14,7	13	167±5,5	15	1	2	6	1	1	2	—	—	—	1	—	—	—	4	6	4	2	1	1	—

*Degree of involvement: I) single tumors up to 1.5 mm in diameter; II) several tumors 2.0-2.5 mm in diameter; III) multiple tumors, largest 5-6 mm in diameter; IV) nearly all surface of intestine occupied by large (often disintegrating) tumors.

†No tumors developed in 2 rats.

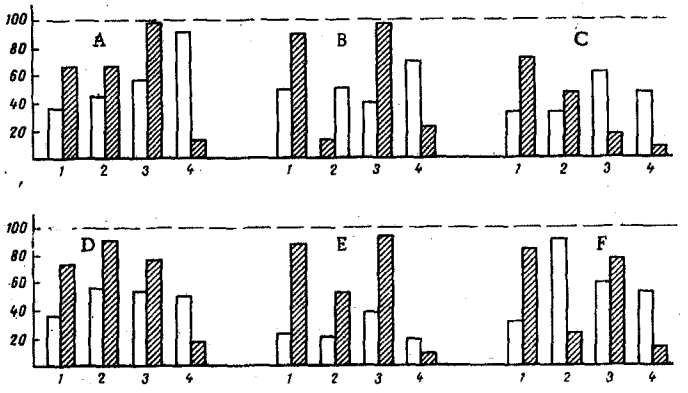

Fig. 2. Radioactivity of macromolecules of various organs depending on rhythm of injection of DMH. A) Liver; B) kidney; C) duodenum; D) ileum; E) ascending colon; F) descending colon. 1) Acid-soluble fraction; 2) protein; 3) DNA; 4) RNA. Ordinate, radioactivity (in % of control level). Unshaded columns – daily injection of DMH in dose of 3 mg/kg (group 1), shaded columns – weekly injection of DMH in dose of 21 mg/kg (group 2).

of the carcinogen tumors developed only in the large intestine and the lesions were less severe. However, in nearly all rats of this group marked degenerative and necrobiotic changes were found in the liver (Fig. 1). Against this background tumors of the liver developed in two cases: a small (0.5 mm in diameter) hepatoma in one case and a hepatoma with well-developed fibrosis, greatly resembling a cholangiocarcinoma, in the other case.

In animals receiving DMH in a dose of 42 mg/kg once every 2 weeks tumors appeared in the same parts of the intestine as with the dose of 21 mg/kg, but the intensity was rather less.

In the experiments to study the binding of metabolites of DMH with the macromolecules of the enterocytes, the radioactivity of nearly all fractions tested in rats of groups 1 and 2 was lower than that of the corresponding samples from the control animals (Fig. 2). Only the level of radioactive label in DNA of the liver and kidneys of the animals of group 2 did not differ significantly from the control. In the acid-soluble fraction radioactivity in the rats of group 1 in all parts of the intestine, and also in the liver and kidneys, was significantly lower than in the rats of group 3, namely 24-47% of its level. Meanwhile its radioactivity in group 2 was much higher than in group 1, and reached 66-89% of the control value. Similar changes in radioactivity were observed in protein and DNA (Fig. 2). The radioactivity of protein in the descending colon and of DNA in the duodenum were exceptions. In these cases the specific radioactivity after daily injection of the carcinogen was higher than after weekly injection, although it remained below the control level.

The pattern was different in the RNA fraction (Fig. 2): in all organs studied the radioactivity was lowest in the rats of group 2 (8-22% compared with the control group), whereas in rats receiving DMH daily it was much higher although did not reach the control level. Meanwhile in the rats of group 2 the RNA content per milligram wet weight of tissue in the various parts of the intestine at the time of injection of the labeled carcinogen was 50-75% lower than in the rats of group 1. The decrease in the specific radioactivity of RNA in the case of weekly injections of the carcinogen can thus be attributed to "dilution of the label."

The results of investigation of the cytochrome P_{450} content in the liver microsomes of the rat showed that after injection of the carcinogen weekly (group 2) its concentration was virtually the same as in the control (5.1 ± 0.589 and 5.32 ± 0.156 mmoles/mg microsomal protein respectively; $P > 0.05$). Meanwhile, daily injection of DMH (group 1) led to a significant ($P < 0.001$) decrease in the content of this enzyme compared with the control (3rd) group (2.48 ± 0.318 mmoles/mg microsomal protein). The cytochrome b_5 content did not depend on the rhythm of injection of the carcinogen.

The decrease in the cytochrome P_{450} content following daily administration of DMH thus evidently reduced the rate of metabolism of the carcinogen. The decrease in the degree of binding of the DMH metabolite with DNA and protein following daily injection of DMH can accordingly be attributed to the delay of its conversion into the proximal metabolite, and this is evidently the cause of the reduced carcinogenic effect in the intestine. After weekly injection of DMH the content of the cytochromes remained virtually unchanged, and as a result the radioactivity of DNA and protein in these animals was high and did not differ significantly from the control (Fig. 2); in turn, this evidently led to intensive formation of neoplasms in the intestine. It is interesting to note that injection of DMH (21 mg/kg) into the animals 2 h before decapitation did not affect the content of cytochromes P_{450} and b_5. Meanwhile, according to the data of Smuckler et al. [9], 2 h after injection of dimethylnitrosamine the content of cytochrome P_{450} is sharply reduced, which the authors cited attribute to the direct action of nitrosamine on the microsomal enzyme. Comparison of these facts suggests that daily injection of DMH leads to inhibition of cytochrome P_{450} synthesis.

The writers showed previously [3] that most of the radioactivity in rats receiving DMH-^{3}H is excreted in the urine in the course of 24 h. Daily injection of DMH leads to a 50% fall in the excretion of radioactivity in the urine during the first 9 h, possible evidence of inhibition of oxidation of the carcinogen. The toxic changes in the liver following daily injection of the carcinogen can be explained by the presence of incompletely oxidized products of DMH metabolism in the body.

Differences in the carcinogenic effect of DMH associated with different rhythms of its administration are thus connected with changes in its metabolism.

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SELECTIVE THERAPY OF LEUKEMIA L1210 BY A COMBINATION OF DEOXYCYTIDINE AND LETHAL DOSES OF CYTOSINE ARABINOSIDE

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Peroral administration of deoxycytidine (dC) to mice with leukemia L1210 simultaneously with intraperitoneal injections of toxic doses of cytosine arabinoside (araC) reduces the severity of toxicosis and prevents the death of the animals by poisoning. A marked antitumor effect is observed in these animals. The mean life span of such mice is much longer than that of untreated mice and also of mice receiving dC or araC alone. With the optimal scheme of treatment about 23% of mice live longer than 60 days. Protection with dC weakens the antileukemic effect of araC when the latter is given in nontoxic doses. This combination is not effective against transplantable myeloid leukemia of mice.

KEY WORDS: leukemia L1210; cytosine arabinoside; 2'-deoxycytidine hydrochloride; selective therapy.

Deoxycytidine (dC) is an antagonist of cytosine arabinoside (araC) and reduces both the severity of its various toxic manifestations and its antitumor effect [1-8]. The writers' observations show that dC, given by mouth to normal mice simultaneously with intraperitoneal injections of araC, weakens the manifestations of the toxicosis and prevents the lethal effect. Analysis of the action of a combination of dC plus araC on the different branches of medullary hematopoiesis shows that the lymphocyte count in the "protected" animals is the same as that observed in mice receiving the antimetabolite alone. This observation suggested that the use of a combination of dC plus araC could lead to selective inhibition or disappearance of lymphoid tumors and leukemias. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Leukemia L1210 was maintained in the ascites form by daily intraperitoneal passage in male DBA/2 mice. In the chemotherapeutic experiments adult female DBA/2 and male BDF_1 hybrids with a body weight of 22-28 g were used as recipients of leukemia L1210. A transplantable strain of myeloid leukemia, obtained in the writers' laboratory from virus-induced Graffi's leukemia was maintained by weekly intravenous passage in C57BL/6j mice. Experiments were carried out on mice of the same strain. All the mice were obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. Cytosar (from Upjohn, USA) and deoxycytidine hydrochloride (from Reanal, Hungary) were used. The compounds were dissolved in physiological saline. In all the experiments treatment began not before the 5th day after inoculation of the mice with tumor cells. The doses, days, and mode of administration of the compounds are indicated in the tables. All compounds were given in a volume of 0.2 ml. dC was given perorally through a curved metal tube. The antitumor

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