EFFECT OF 4-METHYL-2, 6-DI-TERT-BUTYLPHENOL (IONOL) ON INDUCTION OF LIVER TUMORS IN RATS

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Antitumor preparations belonging, for example, to the class of the alkylating compounds are known to possess an anticarcinogenic action [5, 7]. This accounts for the importance of studying the effect of phenolic compounds of low toxicity (inhibitors of radical processes), possessing antitumor activity, on chemical carcinogenesis [1-3]. This problem is even more interesting because it has been postulated in the literature that intermediate free-radical products are formed by the action of carcinogens on the tissues [10], and that in the process of carcinogenesis an EPR signal appears in the liver, possessing a g-factor different from the g-factor of the EPR signal in the normal liver [14].

In the present investigation the effect of 4-methyl-2, 6-di-tert. butylphenol (ionol) on carcinogenesis was studied. The test objects were rats in which liver tumors were induced by including the azo dye p-dimethylaminoazobenzene in the diet. This is a convenient model for studying the inhibition of carcinogenesis because, in contrast to other types of carcinogenesis, limiting the calorie intake does not inhibit carcinogenesis in the liver by azo dyes [13].

EXPERIMENTAL METHOD

The investigations were carried out on male Wistar and August rats initially weighing 140-200 g. Altogether more than 150 animals were used in the experiments. As a first step, in the August rats, the relationship between the incidence of tumor development and the duration of feeding with the carcinogen was established.

The rats received polished rice with 5% corn oil and 0.06% p-dimethylaminoazobenzene, together with carrot. After feeding on the carcinogen for 1, 2, 3, 4, or 5 months, the rats were transferred to a normal diet. The animals were investigated schematically and sacrificed after discovery of tumors. All the rats were sacrificed 12 months after the beginning of feeding.

EXPERIMENTAL RESULTS

The course of development of tumors in the rats' liver is illustrated in the figure. No tumors developed in animals fed with a diet containing the carcinogen for 30-60 days. Feeding for 90 days led to the appearance of tumors in 60% of the animals. When the animals were kept on the diet with the carcinogen for 120 days, 90% developed a tumor of the liver. Feeding with the carcinogen for longer then this did not increase the incidence of tumor development. In rats receiving the carcinogen for 150 days, 12 month after the beginning of feeding 87% showed the presence of a liver tumor.

Prolonged exposure to the carcinogen was thus essential for induction of tumors, namely more than 60 days in these experiments.

The sensitivity of the rats of the two lines used in the experiments to the carcinogen was also compared. The incidence of tumors was lower in the Wistar than in the August rats. When the former were fed with the carcinogen for 150 days, for instance, 65% of them developed a liver tumor within 15 months. The difference in the sensitivity of the two lines of rats to the action of the carcinogen corresponded to a difference in the intensity of the precancerous changes. In the Wistar rats, for instance, slight cirrhosis of the liver was found after 90-150 days. In the August rats the cirrhosis was much more marked, and

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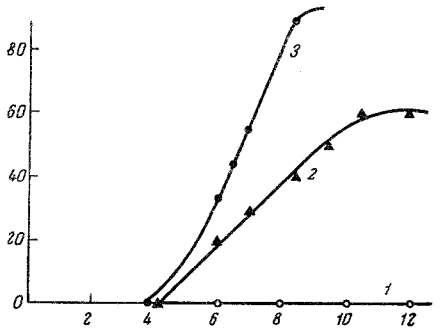

Fig. 1. Development of liver tumors in August rats depending on the duration of action of the carcinogen. Abscissa—time after beginning of feeding with carcinogen (in months); ordinate—percentage of rats with liver tumors. 1) feeding with carcinogen for 30-60 dyas; 2) for 90 days; 3) for 120 days.

frequently whole lobes of the liver were replaced by connective tissue. In the August rats also, foci of cholangiofibrosis, adenomas arising from bile duct cells, and cysts were found.

The tumors arising in most of the animals were single nodules. Occasionally 2 or 3 tumor nodules were observed in different lobes of the liver. Histologically the tumors were of three types: undifferentiated carcinomas, cholangiocarcinomas, and hepatomas. Mixed tumors often appeared.

To study the effect of ionol on carcinogenesis the rats were kept for 150 days on a diet containing 0.06% p-dimethylaminoazobenzene by weight, to which was added 0.3% ionol, also by weight, dissolved in corn oil. None of the 30 Wistar or of the 14 August rats developed a tumor throughout the period of the experiment (12-15 months). In the rats sacrificed after feeding with the carcinogen for various periods practically no precancerous changes were found.

Ionol thus depressed carincogenesis in the rats' liver. To make sure that no direct chemical interaction took place between the p-dimethylaminoazobenzene and the ionol, a 3% solution of the carcinogen in corn oil, containing 15% ionol, was incubated at 37°. After incubation for 7 days the amount of the carcinogen estimated photometrically was only about 15% less than in the 3% solution of p-dimethylaminoazobenzene incubated in the same conditions without addition of ionol. To understand the mechanism of inhibition of carcinogenesis in the liver, it was necessary to study the effect of ionol on the binding of the carcinogen by the proteins of the liver cells. Fixation of a carcinogen by proteins is known to be essential for induction of liver tumors by azo dyes [10] and also, possible, for induction of skin tumors by polycyclic hydrocarbons [4].

The effect of ionol on the fixation of p-dimethylaminozobenzene by the liver proteins was studied in August rats. The animals received p-dimethylaminoazobenzene or p-dimethylaminoazobenzene together with ionol for 30 days. The liver of the rats receiving the carcinogen alone or with ionol was perfused with physiological saline and the amount of dye fixed by the liver proteins was determined by the method of Ward and Spain [15]. In the rats receiving only carcinogen, the amount of dye fixed by the proteins was 22 mμmoles/100 mg protein. In the rats receiving carcinogen and ionol, the amount of the carcinogen fixed by protein was 4 mμmoles/100 mg protein. Ionol thus reduced the amount of carcinogen fixed by the liver proteins by more than five times.

This considereble decrease in the fixation of the carcinogen by protein may be responsible for the inhibition of development of precenacerous changes and for preventing the induction of tumors. Inhibition of the fixation of azo dyes by proteins during treatment with methylcholanthrene led to inhibition of carcinogenesis [12]. It has been shown that methylcholanthrene activates the demethylating enzymes of the liver [11], and the demethylated dye is not fixed by protein and does not cause the development of tumors [10]. It must be determined whether the decrease in the fixation of the carcinogen by protein during administration of ionol, as in the case of methylcholanthrene, is due to the more intensive breakdown of the carcinogen, or whether ionol acts in some other way, such as by preventing the fixation of the unchanged carcinogen by protein.

The view has been expressed [6] that interaction between carcinogens and the cytoplasmic proteins of sensitive cells is the main factor in the mechanism of the carcinogenic action. In this connection the inhibition of fixation of carcinogens by proteins may be regarded as one possible mechansim of anticarcinogenic action.*

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