LIVER TUMORS INDUCED IN MICE BY PRENATAL AND POSTNATAL ADMINISTRATION OF ORTHOAMINOAZOTOLUENE

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The prenatal and postnatal carcinogenic action of orthoaminoazotoluene (OAAT) on the liver was studied in CBA mice. The carcinogen was administered to the mice by the gastric route in oily solution in three doses each of 4 mg (total 12 mg). Tumors of the liver were found 12 months after administration of OAAT in the postnatal period in females in 89.3% of cases compared with 11.5% in the control and in males in 78.7% of cases compared with 68.5% in the control. In the experimental progenies exposed to OAAT during the last 4-5 days of prenatal development tumors of the liver were found in females at the age of 12 months in 54.8% of cases compared with 5.3% in the control and in males in 81.1% of cases compared with 35.2% in the control. In the experimental progenies, especially in males, malignant neoplasms were found more often than in intact animals or those exposed to OAAT in the postnatal period.

KEY WORDS: tumors of the liver; orthoaminoazotoluene; prenatal and postnatal action; transplacental carcinogenesis.

The possibility of transplacental carcinogenesis has now been demonstrated experimentally for various classes of carcinogens, including aminoazo compounds, one of which is orthoaminoazotoluene (OAAT) [1, 2, 5-8, 11]. For this reason the question of age sensitivity of the embryo and the adult to carcinogenic exposure is of considerable theoretical and practical interest. The carcinogenic aminoazo compounds have received least study in this respect. Experiments were carried out in vitro and in vivo to study the effect of OAAT on the liver of embryonic and adult mice. Previous experiments in vitro had shown the high sensitivity of the embryonic mouse liver to OAAT: During organotypic culture of the liver from embryos exposed to OAAT in the prenatal period an increase in the rate of survival of experimental explants was observed compared with the control, and hyperplastic changes developed in them [3, 4]. It was postulated that these changes induced by OAAT in the cultures are precancerous and similar to those observed by the writers previously during the culture of other target tissues exposed to carcinogenic factors in the prenatal period [8]. It was accordingly decided to study the consequences of transplacental action of OAAT on the liver in experiments in vivo.

This paper describes the results of a comparative study of carcinogenesis of the liver in mice exposed to the action of OAAT during prenatal and postnatal development.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice (213 females and 197 males), which are distinguished by a high incidence of spontaneous liver tumors, especially in males, and by sensitivity to the hepatotropic carcinogenic action of chemical carcinogens, especially OAAT. To study the prenatal effect of OAAT on the progeny it was administered to pregnant females 4-5 days before parturition by the gastric route in an oily solution in the form of 3 doses with a total dose of 12 mg per mouse. The progenies of the experimental females were kept with the mothers throughout the period of lactation, after which they were separated. To study the postnatal effect of OAAT of the liver of adult mice, the same doses of the carcinogen were given at the age of 3 months. The experimental mice and their progenies were killed 12 months after administration of OAAT and tumors found on the surface of the liver were measured and counted, after which they were investigated his-

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TABLE 1. Frequency of Appearance of Liver Tumors in Mice Exposed to Prenatal and Postnatal Action of OAAT

Series of experiments	Age of mice investigated, months	Males				Females				
		number of mice tested	number of mice with tumors of liver		index of multiplicity of liver tumors	number of mice tested	number of mice with tumors of liver		index of multiplicity of liver tumors
			absolute	%			absolute	%	
Prenatal exposure to OAAT	12	44	36	81,1	6,4	42	23	54,8	4,9
P				<0,001				<0,001	
Postnatal exposure to OAAT	15	47	37	78,7	4,3	47	42	89,3	9,2
P				<0,01				<0,001	
Control (intact mice)	12	68	42	35,2	1,8	56	3	5,3	1,0
	15	54	37	68,5	2,2	52	6	11,5	2,6

Legend. Values of P given by comparison with intact mice of corresponding age and sex.

tologically in paraffin sections stained with hematoxylin and eosin. Intact CBA mice of the corresponding sex and age (12 and 15 months) served as the control. The χ^2 method was used for statistical analysis of the results.

EXPERIMENTAL RESULTS

On macroscopic examination of the liver of the experimental mice exposed to the prenatal and postnatal action of OAAT, and also of intact mice aged 12-15 months, multiple whitish nodules measuring from 1 to 10 mm in diameter were found. Microscopically these neoplasms were typical benign hepatomas or hepatocellular carcinomas of varied histological structure: trabecular, tubular; anaplastic carcinoma of solid type was the commonest form found. As a rule both benign and malignant tumors of different histological type could be found in the same animal. Besides developed tumors, precancerous changes consisting of diffuse and focal proliferation of hepatocytes were found in the experimental and control mice.

Comparison of the frequency of liver tumors in the intact and experimental mice exposed to a carcinogenic action of OAAT during prenatal and postnatal development showed that it was higher in the experimental groups.

It will be clear from Table 1 that after postnatal exposure of mice aged 3 months to OAAT they developed liver tumors 12 months later, i.e., at the age of 15 months: in 78.7% of cases in males compared with 68.5% in the control and in 89.3% of cases in females compared with 11.5% in the control. Postnatal exposure to OAAT led to a marked increase in the frequency of development of liver tumors evidently only in females. In males the increase was not so pronounced, evidently because of the already high frequency of tumors in intact individuals. However, the difference was statistically significant both for females ($P < 0.001$) and for males ($P < 0.01$).

After administration of the same dose of OAAT to pregnant females their progenies also developed liver tumors at the age of 12 months: in 81.8% in males compared with 35.2% in the control ($P < 0.001$) and in 54.8% of cases in females compared with 5.3% in the control ($P < 0.001$). The dimensions of the tumors and their multiplicity were greater in the experimental mice than in the controls of the corresponding age and sex. For instance, the index of multiplicity of liver tumors in mice receiving OAAT in the postnatal period (F_0) was 9.2 in females and 4.3 in males, compared with 2.6 and 2.2 in the corresponding controls. In mice exposed to OAAT in the prenatal period this index (F_1) was 4.9 in females and 6.4 in males compared with 1.0 and 1.8 in the corresponding controls (Table 1).

Besides liver tumors, adenomas of the lungs also were found in the mice of the groups studied. However, their frequency in the experimental mice was virtually indistinguishable from the control: 20.8-21.1% in females and 11.3-12.9% in males.

Depending on the experimental conditions, the progeny of the experimental mice receiving OAAT in the last third of pregnancy may have been exposed to the carcinogen not only in the prenatal period but also in the early postnatal period, as a result of receiving it through the mother's milk. There are reports in the literature of the induction of tumors in progenies by chemical carcinogens derived from the milk of the experimental females, notably OAAT [1]. However, the carcinogenic effect in the experimental progenies under these circum-

stances was small compared with that which developed in the case of the transplacental action of the carcinogen. With these facts in mind, it can tentatively be suggested that in the present experiments the carcinogenic effect in the experimental progeny was due mainly to the transplacental action of OAAT in the prenatal period.

The results are evidence of the high sensitivity of the liver in embryonic and neonatal mice to carcinogenic factors, for the doses of OAAT given was small compared with those usually used for the experimental production of liver tumors [7]. The scheme used in these experiments for administration of OAAT (3 doses, each of 4 mg, total 12 mg, by the gastric route) enabled benign and malignant hepatomas to be obtained in mice in a large number of cases, i.e., it provides a convenient experimental model of hepatocarcinogenesis.

Comparison of the frequency of appearance of tumors in mice exposed to the postnatal and prenatal action of OAAT showed that in the latter, especially in females, it was lower (54.8%) than in their mothers (89.3%). The lower carcinogenic effect in the progeny may depend on several factors. It may be connected with the fact that the experimental females, at the time of investigation, were 3 months older than their own progeny. During that time the frequency of tumors in the latter may have increased, just as happened in the intact mice (Table 1). This may perhaps be explained by the mechanisms of the carcinogenic action of OAAT and the barrier function of the placenta. Most carcinogenic substances, including OAAT, are known to undergo metabolic activation and detoxication in vivo. As a result, some of the carcinogen may be excreted by the mother without reaching the embryos, for a certain length of time is needed to overcome the placental barrier. As has been shown in the case of polycyclic aromatic hydrocarbons (PAH) the effectiveness of their penetration into embryos and, consequently, the probability of transplacental carcinogenesis, is determined by the permeability of the placenta and the dose and method of administration of the carcinogens to the pregnant female [9, 10]. In particular, after intragastric administration of comparatively small doses of PAH, as was the case in the present experiments with OAAT, much less of the substances passes through the placenta and their accumulation in the fetuses reaches its maximum later than after other methods of administration. Taking all these factors into consideration, in the present experiments a comparatively high carcinogenic effect was observed in the experimental progeny. In the experimental male offspring it was indistinguishable from the effect in males exposed to the carcinogenic action of OAAT in the postnatal period. It must be remembered, however, that unlike in females, a high level of spontaneous liver tumors in males could cancel out the difference in the frequency of induced liver tumors in the groups of mice mentioned above.

Prenatal exposure to OAAT also led to a more malignant course of hepatocarcinogenesis in mice, especially in males. In intact males, for instance, malignant tumors of the liver accounted for 57.9% of all tumors found, in males exposed to OAAT in the postnatal period they accounted for 83.8%, and in males exposed in the prenatal period for 94.3%.

These observations thus demonstrate the high sensitivity of the mouse liver to carcinogenic factors in the prenatal period and, in particular, to administration of OAAT.

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