ONCOGENIC ACTION OF SOME NITROGEN COMPOUNDS ON THE PROGENY OF EXPERIMENTAL MICE

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The oncogenic action of carcinogenic aminoazo compounds (orthoaminoazotoluene and 4-dimethylaminoazobenzene) and cyclic amines (orthotolidine and 3,3'-dichlorobenzidine) was manifested in the progeny of BALB/c mice as a statistically significant increase in the frequency of all tumors in the progeny of the experimental animals compared with those of the control mice. After administration of the carcinogens tumors appeared in new situations, notably leukemias and liver tumors. As a result of the action of noncarcinogenic analogues from the group of aminoazo compounds (para-aminoazotoluene and diethylaminoazobenzene) no significant differences were observed between the progenies of the experimental and control group.

KEY WORDS: carcinogenic aminoazo compounds; cyclic amines; tumors in the progeny; noncarcinogenic analogues.

The possibility that tumors can develop in the progeny of experimental mice treated with oncogenic agents was established in principle about 50 years ago [10, 11, 17]. It is only recently, however, that research in this direction has taken place on a considerable scale, as a result of which many substances have been shown to be capable of exerting a transplacental oncogenic action [1, 5, 13, 14, 16, 19]. Most chemical compounds, however, have not been studied in this respect. In the writers' laboratory systematic studies specifically of transplacental carcinogenesis have been in progress for several years, to examine the behavior not only of well studied oncogenic agents, but also of comparatively neglected chemicals [4, 7, 12].

This paper gives data on the oncogenic effect observed in the progeny of experimental mice receiving carcinogenic aminoazo compounds [orthoaminoazotoluene (OAAT) and 4-dimethylaminoazobenzene (DAB)] and their noncarcinogenic analogues [para-aminoazotoluene (PAAT) and diethylaminoazobenzene (DEAB)], as well as cyclic amines [orthotolidine (OT) and 3,3'-dichlorobenzidine (DCB)] during pregnancy. Whereas the carcinogenic aminoazo compounds have been comparatively well studied and the possibility of their transplacental action has even been demonstrated [2, 8, 9, 15, 20, 21], the cyclic amines have received much less study and no data could be found in the accessible literature on their transplacental oncogenic action [6, 18].

EXPERIMENTAL

Experiments were carried out on BALB/c mice. In the last week of pregnancy the animals were given four or five subcutaneous injections of the substances listed above, dissolved in sunflower oil. OT and DCB were given in doses of 2 mg per injection in 0.1 ml sunflower oil, i.e., the animal received a total of 8-10 mg of the compound. The remaining substances (OAAT, PAAT, DAB, DEAB) were injected in doses of 24 mg in 0.2 ml sunflower oil per injection, i.e., the total dose was 96-120 mg per mouse.

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TABLE 1. Oncogenic Action of Some Nitrogen Compounds on the Progeny of BALB/c Mice

Compound	Number of mice	Number of mice with tumors			Number of tumors of different types									
					tumors of the lungs		hepatomas		mammary tumors		leukemias		other tumors	
		abs.	%	P	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Control	30 (19♀+11♂)	6	20		3	10	—	—	3	15,7	—	—	—	—
DAB	25 (10♀+15♂)	14	56	0,01	8	32	3	12	7	70	2	8	2	8
DEAB	24 (16♀+8♂)	4	16,6	0,05	3	12,5	—	—	3	18,7	—	—	—	—
OAAT	41 (19♀+22♂)	24	58,5	0,001	15	36,5	5	12,1	8	42,1	9	21,9	3	7,3
PAAT	25 (18♀+7♂)	4	16	0,05	1	4	—	—	3	16,6	—	—	—	—
OT	16 (7♀+9♂)	8	50	0,05	6	37,5	—	—	5	71,4	—	—	—	—
DCB	24 (11♀+13♂)	13	54,1	0,01	5	20,8	—	—	4	36,3	7	29,1	—	—

[1]The percentage of mammary gland tumors was obtained by dividing their number by the number of females.

[2]Among the other tumors, one cavernous hemangioma and one myoma were observed after treatment with DAB and one myoma and two intestinal tumors after treatment with OAAT.

The progeny was kept together with the experimental animals throughout the period of lactation and taken from them at the age of 3-4 weeks. Many of the progeny of the experimental animals died during the first few days after birth. Observations continued on the surviving mice until their natural death. Most of them survived for 18-20 months, some died at the age of 10-12 months. Altogether 155 experimental animals (81 females and 74 males), the progeny of 78 experimental mice, were studied. The control group consisted of intact mice born from intact females and fed by them.

The mice which died were examined macroscopically and sections were cut from their organs. The results were subjected to statistical analysis by the Student–Fisher method. The results are summarized in Table 1.

RESULTS

As Table 1 shows, the control group of animals gave a low frequency of mammary gland tumors (15%). In their morphological structure they were adenomas. Tumors of the lungs appeared in 10% of cases and were typical mixed glandular and papillary adenomas as frequently described [3, 4].

Tumors were found in 14 of 25 cases in the progeny of the mice receiving DAB, or 56% ($P < 0.001$). Just as in the control, adenomas of the lungs (32%; $P < 0.05$) and tumors of the mammary glands (70%; $P < 0.001$) were observed in these animals. In another three cases tumors of the liver, consisting of single and multiple nodules, were found. On microscopic investigation they were hepatomas of the trabecular type, with a varied degree of atypism. Lymphatic leukemias were found in two cases. Examination of the progeny of the mice receiving the noncarcinogenic analogue, DEAB, revealed no significant differences from the control (Table 1).

Injection of OAAT into the pregnant mice also induced tumor development in their progeny. Tumors were found in 24 of 41 experimental mice, i.e., in 58.5% of cases ($P < 0.001$). A considerable increase in the number of tumors compared with the control also was observed in this series of experiments. For instance, 36.5% of cases ($P < 0.01$) compared with 10% in the controls, and mammary gland tumors in 42.1% ($P < 0.01$) compared with 15% of cases. As in the preceding series, the lung tumors as a rule were multiple adenomas of different sizes and of the tubular or tubulo-papillary type. In four cases, however, adenocarcinomas were found. Tumors of the mammary glands were adenocarcinomas.

Just as after treatment with DAB, OAAT led not only to an increase in the number of "spontaneous" tumors, but tumors also appeared in new situations. In five cases, for example, tumors developed in the liver. These were hepatomas, single and multiple. Three of them, moreover, were found in animals dying at the age of 12 months. Leukemias were observed in nine mice (21.9%; $P < 0.01$): lymphatic leukemia in five animals and reticulosis in four.

Examination of the progeny of mice receiving the noncarcinogenic analogue, PAAT, during pregnancy revealed no significant differences from the control group (Table 1).

Administration of cyclic amines to the pregnant animals also led to a substantial increase in the frequency of tumors in the experimental progeny compared with the control. After treatment with OT, half of

the 16 mice studied were found to have tumors: adenomas of the lungs in six (37.5%; $P < 0.01$) and tumors of the mammary glands in five (71.4%; $P < 0.001$).

Under the influence of DCB tumors developed in 13 of the 24 mice (54.1%; $P < 0.01$). The tumors were adenocarcinomas of the mammary glands in four mice (36.3%; $P > 0.05$), adenomas of the lungs in five mice (20.8%; $P > 0.05$), and lymphatic leukemia (absent in the control) in seven mice (29.1%; $P < 0.01$).

The investigation thus demonstrated a statistically significant increase in the total number of tumors in the experimental progeny (compared with the intact control) after administration of carcinogenic aminoazo compounds (OAAT and DAB) and cyclic amines (OT and DCB). It must be emphasized that after treatment with noncarcinogenic analogues of the group of aminoazo compounds (PAAT and DEAB) no significant differences were observed between the progeny of the experimental mice and the control group. After treatment with the carcinogens tumors also appeared in new situations, primarily tumors of the liver and leukemias. Leukemias were observed under the influence of all the oncogenic agents except OT. Hepatomas appeared only after treatment with the hepatotropic agents OAAT and DAB.

Because of the scheme chosen for the experiments the observed increase in the number of tumors in the experimental animals could have been caused in another way than by the transplacental action of these substances. Being excreted from the body comparatively slowly, they could be transmitted through the milk of the lactating mothers. This possibility has been demonstrated experimentally both for aminoazo compounds [2, 9] and also for other carcinogenic substances [13]. In the experiments described above, the action of the substances was evidently combined – transplacental and postnatal – in the early and very sensitive period of life.

The results of the experiments especially with the cyclic amines, which are used in some cases in the aniline dye industry, make it imperative to take into consideration the possible carcinogenic action of these compounds both on persons in direct contact with them, and on their progeny (transplacental transmission, transmission through the milk of lactating mothers).

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