SOME ASPECTS OF TRANSPLACENTAL CARCINOGENESIS IN RABBITS

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The transplacental carcinogenic action of various known carcinogens – ethylnitrosourea (ENU), methylnitrosourea (MNU), dimethylbenzanthracene (DMBA), and benzo(a)pyrene (BP) – was investigated in experiments on rabbits. ENU was found to have the strongest carcinogenic activity, with a well-marked neurotropic effect. It was shown at the same time that the relative affinity for various tissues that is characteristic of the action of certain carcinogens on the adult organism is not always manifested in transplacental carcinogenesis. The study of the effect of various modifying factors (organ-specific immunization, chronic peripheral nerve stimulation, injection of MNU) on the realization of transplacental carcinogenesis showed that their postnatal administration mainly stimulates the development of peripheral nerve tumors in the progenies (MNU inhibits tumor development).

KEY WORDS: transplacental carcinogenesis; carcinogens; modifying factors.

Special interest has recently been aroused in the study of sensitivity of different species of animals to the transplacental carcinogenic action of certain known carcinogens and the effect of modifying factors on tumor development. Druckrey et al. [7] first found that ethylnitrosourea (ENU), if injected intravenously into pregnant rats, caused tumors of the nervous system in their progeny. If this carcinogen was injected into hamsters, multiple neoplasms of the peripheral and cranial nerves developed in the progenies [12, 14], whereas adenomas of the lungs and leukemias developed in mice [4, 6]. Napalkov and Aleksandrov [5] found that after injection of dimethylbenzanthracene (DMBA) into pregnant rats tumors of the nervous system and kidneys are selectively induced in their progenies. As a result of the transplacental action of methylnitrosourea (MNU), a powerful neurotropic carcinogen for adult rats, mainly tumors of the kidneys and nervous system, appear in their progenies. Nevertheless, the question of differences in the pattern of development of neoplasms induced by transplacental action of different carcinogens has been inadequately studied. There are isolated reports [8] of the production of kidney tumors in the progeny of pregnant rabbits after administration of ENU. Data on the role of modifying factors in the development of the phenomenon of transplacental carcinogenesis are particularly scanty.

Accordingly, in the investigation described below transplacental carcinogenesis in rabbits was studied after administration of various carcinogens and the effect of some modifying factors on tumor development was examined.

EXPERIMENTAL METHOD

Experiments were carried out on 110 pregnant chinchilla rabbits, divided into two series. The animals of series I (60 rabbits) received an intravenous injection of various carcinogens on the 25th-26th day after fertilization: ENU in a dose of 80 mg/kg (group 1, 12 animals), MNU in a dose of 40 mg/kg (group 2, 12 animals), DMBA in a dose of 20 mg/kg (group 3, 12 animals), and benzo(a)pyrene (BP) in a dose of 30 mg/kg (group 4, 12 animals); 12 pregnant rabbits receiving an intravenous injection of physiological saline served as the control. In series II 50 pregnant rabbits received an intravenous injection of ENU in a dose of 80 mg/kg on the 25th-25th day after fertilization. The progenies of these rabbits were divided into four groups, with 36 animals in each group. The animals of group 1 were immunized 3 times, at intervals of 10 days, with antigen from homologous rabbit sciatic nerve tissue (together with Freund's complete adjuvant). A focus of chronic irritation was created in the rabbits of group 2 by tying a nylon ligature around the left sciatic nerve; the animals of group 3 were given an intravenous injection of MNU once a week in a dose of 10 mg/kg for 15 months.

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TABLE 1. Frequency of Tumor Development and Mean Survival Period of Progenies of Rabbits following Transplacental Action of Various Carcinogens

Carcinogen tested	Number of animals in experiment					Mean survival period of rabbits with tumors, days	
	until day of taking from mothers	surviving until time of appearance of first tumor	frequency and site of induction of tumors				
			in all localizations	of peripheral nervous system	of kidneys	of peripheral nervous system	of kidneys
ENU	36	33 (170 and 160)	16	8	4	437,8±24,3	326,5±13,1
MNU	38	31 (160 and 150)	6	1	4	622±16,4	286,5±14,7
DMBA	35	32 (160 and 160)	7	4	2	600,5±21,3	334,5±7,4
BP	36	30 (180 and 120)	5	3	2	642,5±10,1	372±16,3
Control	38	34 (180 and 160)	—	—	—	—	—
Total	183	160	34	16	12		

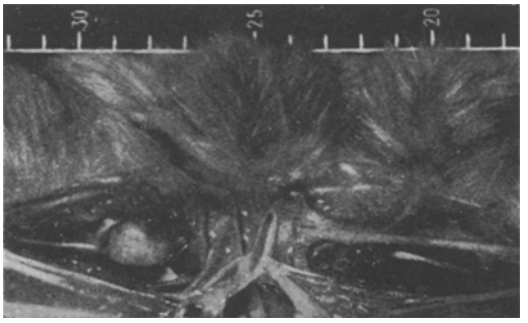

Fig. 1. Neurinoma of left sciatic nerve in offspring of a rabbit receiving organ-specific immunization.

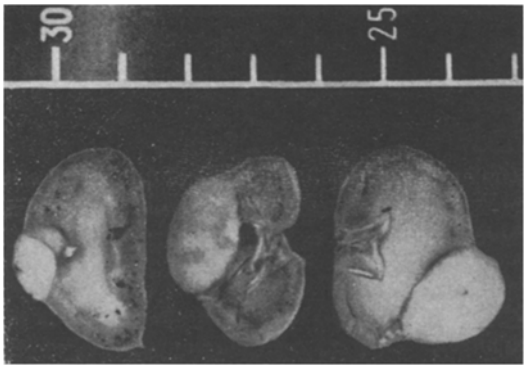

Fig. 2. Kidney tumors induced in rabbits during postnatal administration of various modifying factors.

EXPERIMENTAL RESULTS

Analysis of the data shows that the rabbit placenta is not a barrier for polycyclic aromatic hydrocarbons and nitroso compounds and that administration of these carcinogens in the last third of pregnancy leads to induction of tumors, predominantly of peripheral nerves and the kidneys, in their progenies. The comparative study of the transplacental action of ENU, MNU, DMBA, and BP showed convincingly that ENU has the strongest carcinogenic activity in rabbits; it had a very well-marked neurotropic carcinogenic action (Table 1).

TABLE 2. Distribution of Transplacental Tumors in Rabbits Depending on Sex, Location, and Influence of Modifying Factors

Modifying factors	Number of animals and time of appearance of tumors	Tumors								Mean latent period of appearance of peripheral nerve tumors, days
		central nervous system	peripheral nervous system		kidneys		other locations		total	
		♀	♂	♀	♂	♀	♂	♀		
Immunization with antigen from homologous sciatic nerve tissue	27	1	2	6	1	3	2	2	17	463,5±40,2
Chronic irritation of sciatic nerve	26	—	4	3	1	2	2	—	12	474,1±27,2
Intravenous injection of MNU	29	—	1	1	—	1	—	1	4	721±23,1

MNU, a powerful carcinogen with distinct affinity for nerve tissue in adult rats, when administered experimentally over long periods [2, 7, 9], had a weaker transplacental carcinogenic action than EMU. Administration of MNU in the last third of the gestation period caused the formation predominantly of kidney tumors in the surviving rabbits. Following a single injection of DMBA into pregnant rats, tumors mainly of the mammary gland develop; the results now obtained confirmed that administration of DMBA and BP to pregnant rabbits leads to the formation of peripheral nerve and kidney tumors in their progeny. The present results are evidence that the characteristic relative affinity of certain carcinogens for particular organs and tissues when acting on the adult organism is not always manifested in transplacental carcinogenesis.

According to data in the literature, modifying factors have a marked stimulating action on transplacental carcinogenesis in rabbits. Statistical analysis of the data by the χ^2 criterion showed a definite effect of modifying factors on the frequency of tumors induced by ENU and of neurogenic neoplasms in particular. The factors used also had a clear effect on the times of appearance of the first peripheral nerve tumors. The development and manifestation of the tumor process were shown to take place more rapidly and easily against the background of various changes in reactivity of the animal and in the course of chronic irritation (Table 2).

Injection of MNU, a resorptive carcinogen with definite affinity for tissues of the nervous system [1, 3, 5, 7, 8, 11, 12], was found to inhibit tumor growth in the progenies of the rabbits and to prevent transplacental carcinogenesis induced by ENU. Similar results were obtained by Pezzetta et al. [13] in rats, after injection of various nitrosourea derivatives into the progenies of rabbits. Chronic irritation of the sciatic nerve was found to have a stimulating effect on the onset and development of transplacental tumors of peripheral nerves in the progenies of the rabbits, in full agreement with clinical observations when various forms of trauma in children lead to the rapid appearance of tumors in the nervous system.

The results of these investigations confirm that organ-specific immunization with antigen from homologous peripheral nerve tissue leads to stimulation of transplacental carcinogenesis in rabbits, evidently in connection with changes in the reactivity of the animal and, in particular, with changes in autoimmune and autoallergic processes as a result of immunological action, with the consequent modification of resistance of the progeny of the rabbits to induction and growth of transplacental tumors. It is interesting to note that under these circumstances, besides an increase in the frequency of peripheral nerve tumor formation and shortening of the minimal latent period of their development, changes also take place in the morphological structure of transplacental kidney tumors. The fact that sympathoblastomas of the kidneys were obtained in the progenies of rabbits confirms Masson's opinion [10] that both the nephrogenic anlage and the primitive neuroepithelium may play a part in the formation of the neuroectodermal variant of embryonic nephroma (Wilms' tumor).

The results of these experiments thus show that the postnatal application of various modifying factors influences transplacental carcinogenesis in rabbits caused by injection of ENU during pregnancy and facilitates the induction in their progenies of tumors that are most frequently encountered in children.

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HEMATOPOIETIC TISSUE REACTIONS ASSOCIATED WITH GROWTH OF SYNGENEIC TRANSPLANTABLE TUMORS IN MICE

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Growth of a hemangiopericytoma in syngeneic (CBA× C57BL/6j)F_1 male mice led to regular changes in hematopoiesis: an increase in the weight and number of the spleen cells, an increase in the number of colony-forming units (CFU) in the spleen, intensification of myelopoiesis in the spleen, and the development of leukocytosis with a sharp increase in the number of polymorphonuclear granulocytes in the circulating blood, i.e., a "leukemoid reaction" syndrome. This syndrome developed also when tumor cells were injected into a splenectomized host. A leukemoid reaction, although less marked, also was found in the late stages of development of a hepatoma, transplanted into syngeneic (CBA× C57BL/6j)F_1 male mice. Meanwhile, transplantation of a syngeneic strain of carcinoma of the bladder into the same mice did not lead to the development of a leukemoid reaction.

KEY WORDS: hemangiopericytoma; hepatoma; carcinoma of the bladder; leukemoid reaction.

During growth of certain solid tumors in animals and man changes in hematopoiesis – the so-called leukemoid reaction – have frequently been described [4, 5, 10, 12]. Leukemoid reactions in mice were characterized by a high leukocytosis, with the dominance of polymorphonuclear neutrophils, splenomegaly, an increase in the number of stem cells in the spleen and bone marrow, and extramedullary hematopoiesis in the liver, kidneys, and lungs [1, 3, 7-9].

The object of the present investigation was to study the leukemoid reaction in mice with transplantable tumors of strains of hemangiopericytoma, hepatoma, and carcinoma of the bladder. The strains were obtained in the writers' laboratory and maintained by serial passage through syngeneic recipients [2].

EXPERIMENTAL METHOD

Experiments were carried out on adults (CBA× C57BL/6j) mice weighing 20-22 g reared at the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. The mice were divided into two groups: the mice of group 1 were inoculated with tumors, the mice of group 2 (control) remained intact. Tumors were transplanted subcutaneously into male mice: hemangiopericytoma, $1 \cdot 10^6$ cells per animal; carcinoma of the bladder

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