TRANSPLACENTAL CARCINOGENIC ACTION OF p-HYDROXYPHENYL-LACTIC ACID

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Through the transplacental action of the tyrosine metabolite p-hydroxyphenyl-lactic acid, which has carcinogenic activity, on progeny of CC57BR and C57BL mice, malignant and benign neoplasms (leukemias, lymphosarcomas, adenomas and carcinomas of the lung, hepatomas, papillomas, carcinomas of the bladder, and other tumors) developed in 88 and 78% of cases respectively. The number of these neoplasms was significantly higher than in the control, they appeared sooner, and they were more malignant in character.

KEY WORDS: transplacental action; p-hydroxyphenyl-lactic acid; malignant and benign tumors.

Reliable experimental evidence of the role of transplacental transmission of exogenous carcinogens has now been obtained [1, 2, 6, 7, 9, 11, 12, 14, 15].

Meanwhile the transplacental action of endogenous substances such as carcinogenic tryptophan metabolites and tyrosine derivatives is also a possibity. This suggestion is based on the known presence of 3-hydroxyanthranilic acid and 3-hydroxykynurenin (carcinogenic metabolites of tryptophan) in women with toxemias of pregnancy, probably resulting from vitamin B_6 deficiency [13]. Cases of congenital leukemia have been described in children whose mothers, clinically healthy women, have been found to have disturbances of tryptophan metabolism and to excrete 3-hydroxykynurenin and 3-hydroxyanthranilic acid in their urine [5, 8]. Finally tryptophan and tyrosine metabolites have a low molecular weight so that they readily pass through the placenta.

The object of this investigation was to study the transplacental carcinogenic action of the tyrosine metabolite p-hydroxyphenyl-lactic acid (p-HPLA), which possesses marked carcinogenic and leukemogenic activity [3].

EXPERIMENTAL METHOD

Experiments were carried out on CC57BR and C57BL mice of both sexes. The compound p-HPLA was injected subcutaneously into pregnant animals in two variants: during the first 10 days (from the first day after discovery of a vaginal plug) or during the last week of pregnancy, in a dose of 5 mg in 0.25 ml distilled water daily. The total dose of the preparation in the first variant was 50 mg and in the second 20-30 mg per mouse. The newborn mice were weaned after the usual period of feeding and remained under observation for 22 months. At birth and during maternal feeding the animals were examined for congenital deformities. Young mice, both experimental and control, which died also were examined for congenital deformities and the dimensions of their trunk and limbs were measured.

The experimental animals in which tumors or leukemias were discovered were killed when the disease was established; the remaining animals were killed at the age of 17-22 months. The control animals also were killed at the age of 17-22 months.

All killed and dying animals were autopsied.

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TABLE 1. Carcinogenic Action of p-HPLA on Progeny of Mice Receiving It during Gestation

Line of mice	No. of mice surviving appearance of 1st neoplasm	No. of mice with neoplasms: absolute	No. of mice with neoplasms: %	Age of mice at appearance of 1st neoplasm, mos.	No. of neoplasms per mouse	Type of neoplasms: hemoblastoses: lymphatic leukemia	hemoblastoses: lymphosarcoma	adenomas: of lung	adenomas: of other organs	carcinoma of lung	papilloma of bronchus	hepatoma	hemangiomas: of liver	hemangiomas: of other organs	papilloma of bladder	carcinoma of bladder	myosarcoma	sarcoma	other tumors
CC57BR	160	141	88	9	1,9	85	16	58	4	9	2	37	13	9	16	9	1	3	1
Control	26	10	38	17	1,0	5	—	3	—	—	—	—	2	—	1	—	—	—	—
C57BL	74	58	78	8	1,2	42	12	5	—	—	2	4	—	—	3	—	1	1	3
Control	29	4	14	17	1,0	2	1	—	—	—	—	—	1	—	1	—	—	—	—

Cytochemical tests were carried out on tumor cells and on squash preparations of the spleen and lymph nodes of some animals with tumor-like leukemia to make a differential diagnosis between lymphosarcoma and reticulosarcoma.

EXPERIMENTAL RESULTS

In all the experimental animals, including mice killed or dying during the first days after birth and also at other times, no congenital deformities of any kind could be found. Presumably the dose of p-HPLA (30 mg), injected during the first three days after the beginning of pregnancy, i.e., before formation of the placenta, was insufficient to allow the p-HPLA to exert a teratogenic action. It must be emphasized that the dose of p-HPLA used in these experiments corresponded quantitatively to the levels of this metabolite determined in patients with leukemia [4].

Cases of congenital deformities in man associated with disturbances of tyrosine metabolism manifested as tyrosyluria likewise are unknown. These two pieces of evidence thus together indicate that p-HPLA has no teratogenic properties.

The results of investigations of the transplacental carcinogenic action of p-HPLA are given in Table 1. They show that malignant and benign tumors developed in 88% of the progeny of the CC57BR mice. The group of malignant tumors consisted of leukemias and lymphosarcomas, carcinoma of the lung and bladder, and sarcomas. A high proportion of the animals (37) developed hepatomas; in one case metastasization of the tumor in the lung was observed. Often adenomas of the lungs and papillomas of the bladder and also hemangiomas of the liver and other organs were found in the animals. Papillomas of the bronchus were found in two mice.

In the progeny of the C57BL mice, neoplasms developed in 78% of cases (Table 1). Most neoplasms in the mice of this strain were hemoblastoses, and some animals had hepatomas, myosarcoma, and sarcoma.

Cytological diagnosis of the tumor-like forms of leukemias in the mice was supplemented by various cytochemical tests indicating the histogenesis of the disease. Squash preparations of lymph nodes, spleen, and liver were investigated in 23 animals with the diagnosis of lymphosarcoma or lymphatic leukemia. Activity of acid and alkaline phosphatases, α-naphthylacetate esterase, chloresterase, and peroxidase and the polysaccharide and RNA concentrations were studied in squash preparations of the organs. Activity of acid phosphatase and α-naphthylacetate esterase inhibited by sodium fluoride and potassium–sodium tartrate also was studied. In all animals with a diagnosis of lymphatic leukemia and lymphosarcoma (diffuse forms — lymphocytic, prolymphocytic) a weak granular reaction was observed for polysaccharides, acid phosphatase activity was moderate in 25-30% of cells whereas in the rest it was weak or completely inhibited on the addition of potassium–sodium tartrate to the medium. Esterase activity in these cells was moderate and was not inhibited by NaF; Brachet's test was weakly positive.

The results of the cytochemical analysis thus showed that all cases of tumor-like leukemias were lymphosarcomas.

On the whole, the indices of carcinogenic activity following the transplacental action of p-HPLA on mice of both lines was significantly higher than the incidence of spontaneous tumors in mice of these strains. Carcinogenesis induced by p-HPLA was characterized by a high frequency of malignant neoplasms and by earlier times of development of the tumors. For

instance, whereas the control animals began to develop spontaneous tumors after the age of 17 months, in the experimental mice tumors were found at the age of eight to nine months. In the control mice only one case of lymphosarcoma was noted, whereas in the experimental mice this type of hemoblastosis was found from six to eight times more frequently.

Carcinoma of the lung and bladder developed in the experimental animals but never in control animals under observation until the age of 22 months. No case of development of hepatoma likewise was found in the control animals, whereas among the experimental mice, especially the CC57BR strain, this type of neoplasm was found fairly often.

The number and histogenesis of tumors and leukemias developing as a result of transplacental action corresponded, it will be noted, to these indices as found previously in a study of the action of p-HPLA on adult animals [3].

The results of these investigations, in the writers' view, are important in connection with the discussion of the possible etiological importance of endogenous carcinogens in the development of congenital and infantile leukemias.

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