TRANSPLACENTAL ACTION OF ORTHOAMINOAZOTOLUENE ON ORGAN CULTURES OF EMBRYONIC LIVER OF C57BL AND CBA MICE

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The transplacental action of orthoaminoazotoluene (OAAT) was studied in organ cultures of embryonic liver from CBA mice with a high incidence of hepatoma, and C57BL mice with a low incidence of cancer. In response to the transplacental action of 12 mg of OAAT, increased survival of the embryonic liver cultures of both strains of mice was observed. In response to the transplacental action of 24 mg of OAAT on CBA mice, a toxic effect was observed in the early stages of culture, but by the 20th-25th day of culture this was replaced by a growth-stimulating action of the carcinogen. The results of the experiments in vivo correlated with the observations in vitro.

KEY WORDS: organ culture; embryonic liver, transplacental action; orthoaminoazotoluene.

Many carcinogens belonging to different classes of chemical compounds are known to produce tumors in various situations in the progeny of experimental animals by transplacental action. Such carcinogens include orthoaminoazotoluene (OAAT) [1, 8]. Organ cultures of embryonic tissues are a convenient model with which to study transplacental action [2, 5, 7].

This paper describes a comparative study of organ cultures of embryonic liver from intact C57BL and CBA mice and from mice exposed to the transplacental action of OAAT.

EXPERIMENTAL METHOD

Starting from the 16th day of pregnancy female C57BL mice were given OAAT by mouth in 0.15 ml of sunflower oil, three times, in a total dose of 12 mg. The CBA mice received OAAT under the same conditions in total doses of 12 and 24 mg. On the 19th-20th day of pregnancy the experimental females were killed and the livers of the embryos were explanted into organ culture. Cultures of the livers of intact C57BL mice at the same stage of embryonic development acted as the control. The livers were cultured in nutrient medium by Luria's method [6] in the modification adopted in the present writer's laboratory [5]. Culture continued for 34-37 days. The explants were studied at various stages of the experiment in total preparations, obtained after fixation of the cultures in 70-80% ethanol and staining with hematoxylin. The statistical analysis of the results was carried out by the χ^2 method.

EXPERIMENTAL RESULTS

The morphological picture of the organ cultures of normal embryonic liver of CBA mice was described in detail by the writer previously [5]. No significant morphological differences were found in the organ cultures of embryonic liver from C57BL mice.

The main processes observed during culture of the embryonic liver were the formation of an epithelial zone of growth around the explants, which appeared as early as on the 4th-5th day, and reached its maximum

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TABLE 1. Transplacental Action of OAAT on Organ Cultures of Embryonic Liver of C57BL and CBA Mice

Duration of culture, days	C57BL mice							CBA mice									
	control			transplacental action of OAAT (12 mg/mouse)				control			significance of difference between control for C57BL and CBA	transplacental action of OAAT					
												12 mg/mouse				24 mg/mouse			
	No. of explants studied	No. of living explants		No. of explants studied	No. of living explants		P	No. of explants studied	No. of living explants			No. of explants studied	No. of living explants		P	No. of explants studied	No. of living explants		P
		abs.	%		abs.	%			abs.	%			abs.	%			abs.	%	
4—5	16	9	56,2					152	77	50,6	<0,001	49	48	98	<0,1	116	19	16,4	<0,001
6—9	41	39	95,2	26	23	92	>0,1	120	85	70,7	<0,01	34	33	97	<0,05	40	16	40,0	<0,001
10—13	14	14	100	42	38	90,5	>0,1	120	81	67,5	<0,05	14	14	100	<0,1	29	20	69,0	>0,1
14—17	14	8	57,1	16	16	100	<0,01	191	119	62,2	>0,1	27	26	96,3	<0,01	115	76	69,1	<0,001
19—20	13	5	38,4	14	8	57,1	<0,1	54	32	59,3	<0,05	21	21	100	<0,01				
22—25	21	6	28,6	21	15	71,4	<0,01	135	43	31,9	>>0,1					35	15	43,0	<0,001
27—29	14	9	64,2	28	17	60,7	>0,1	46	23	50	>0,1	15	15	100	<0,01				
31—34	13	6	46,1	30	25	83,2	<0,05	11	4	36,3	>0,1	14	7	50	>0,1				
37												24	10	41,7					

of development by the 10th-12th day. At the same time some flattening of the explant took place, as shown by the gradual transition from the stratified center of the explant to the monolayer of the peripheral membrane. Intensive hematopoiesis was observed, as separate foci, until the 16th-17th day. Individual blood cells, liver epithelial cells, and macrophages migrated outside the limit of the explants. A central necrotic focus, later replaced by epithelial tissue, was found in the center of the fragment after the first days of culture.

During assessment of viability of the cultures, taking all these processes into account, attention was concentrated on the development of the epithelial zone of growth.

The survival rate of the control cultures of embryonic liver from intact C57BL mice was higher than that of CBA mice. This was noticeable as early as on the 6th-9th day of culture, when the number of viable C57BL explants was 95.2%, rising to 100% by the 10th-13th day, whereas the number of viable CBA explants on the 6th-9th day was only 70.7%, and later their number began to fall gradually to 36.3% by the 31st-34th day.

The morphological picture and survival rate of the experimental cultures of embryonic liver of C57BL mice exposed to the transplacental action of 12 mg of OAAT was indistinguishable from the control until the 13th day (Table 1). From the 14th-17th day the control cultures began to die gradually, and by the 31st-34th day of culture the number of living explants was 46.1%. The survival rate of the experimental cultures throughout this period remained almost at the same level, and by the 31st-34th day 83.2% of the explants were viable, which was 37% higher than the level of survival in the control ($P < 0.05$). In the experimental cultures of embryonic liver from CBA mice, the same dose of OAAT (12 mg/mouse) considerably stimulated the development of this zone of growth at the first periods of culture (4th-5th day) in 98% of explants and this high survival rate was maintained until the 29th day. The percentage of living control cultures of embryonic liver of CBA mice did not exceed 70.7 at any time during culture, and by the 29th day it had fallen to 50.

The carcinogen, in a dose increased to 24 mg/mouse had a strong toxic action in embryonic liver cultures of CBA mice, which was observed during the first 9 days of culture and was manifested as depression of the formation of the epithelial zone of growth around the explants. For instance, on the 4th-5th day there were significantly fewer viable explants in the experimental series (16.4%) than in the control (50.6%). On the 6th-9th day the number of these explants in the control had increased to 70.7%, compared to only 40.0% in the experimental group. Later in the course of cultivation an increase in survival was observed among the experimental cultures, whereas it was reduced in the control. This difference became particularly marked on the 22nd-25th day of culture, when the number of viable explants in the experimental series was 43% and in the control 31.9% (Table 1).

In the experiments in vivo transplacental exposure to OAAT in a dose of 24 mg/mouse also had a strong toxic action. For instance, after administration of this dose to 45 pregnant CBA mice, in no case was viable progeny obtained, whereas after a dose of 12 mg given to 50 pregnant mice, all the young were viable. These observations in vivo correlate with those in vitro, especially at the early times of culture.

This comparative study of the survival rate of organ cultures on normal embryonic liver from two strains of mice thus showed that it is higher in C57BL mice. The OAAT stimulated growth of the cultures in

the early stages and increased their viability in the last stages of cultivation. This effect was manifested more clearly in the experimental cultures of embryonic liver from CBA mice, whose survival rate under normal conditions was lower than that of C57BL. Other workers have observed the same effect in organ cultures of embryonic liver and kidney tissues [3, 4, 7] during the transplacental action of various carcinogens, whereas the transplacental exposure to pyrene, the noncarcinogenic analogue of benzo(a)pyrene did not increase the survival rate of organ cultures of the lungs [2].

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PHARMACOKINETICS OF FTORAFUR-2-^{14}C IN RATS WITH WALKER'S CARCINOSARCOMA

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The pharmacokinetics of ftorafur-2-^{14}C (FF) after intravenous injection was investigated in experiments on rats with Walker's carcinosarcoma. The level of FF and its metabolites in the blood plasma was shown to fall in the manner of a three-phase process. The concentration of the compound in the tissues falls in the order: kidneys, small intestine, tumor, stomach, muscles, heart, liver, lungs, spleen, brain, and fat. The presence of FF-2-^{14}C and its metabolite, endogenous 5-fluorouracil, was observed in the tumor. Excretion of the compound continued for 48 h, 52.2% being excreted through the kidneys, 38% through the lungs, and 0.8% of the injected dose with the feces.

KEY WORDS: ftorafur; 5-fluorouracil; permeability; metabolism; Walker's carcinosarcoma.

The ability of ftorafur-2-^{14}C (FF) and its metabolite 5-fluorouracil-2-^{14}C (5-FU) to penetrate into tumor tissue was investigated and the length of stay of the compound in the body of animals with tumors was estimated.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 170-200 g were used. Ftorafur-2-^{14}C had a specific radioactivity of 2.8 μCi/mg. The compound was given intravenously as a single dose of 150 mg/kg (11 μCi/kg). The animals were killed 0.25, 0.5, 1, 2, 3, 6, 10, and 24 h after injection of the compound and the radioactivity was determined in the blood and various organs with the SL-30 (Intertechnique) scintillation counter with correction of the

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