TRANSPLACENTAL BLASTOMOGENIC ACTION OF URETHANE AT DIFFERENT STAGES OF POSTNATAL LIFE OF MICE

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A study of the transplacental blastomogenic action of small, medium, and large doses of urethane at various stages of postnatal life of line A mice shows that in animals aged 2-3 weeks there is a definite relationship between the blastomogenic effect and dose of urethane received through the placenta. Later, by the age of 10-19 weeks, the incidence of adenomas of the lungs in mice becomes approximately the same as a result of the action of small and medium doses of urethane, although the latent period of tumor development varies. Embryonic mouse lung tissue is highly sensitive to the action even of small doses of urethane, the blastomogenic effect of which, while difficult to detect in the early period of postnatal life, subsequently becomes equal to the blastomogenic effect of larger doses of this substance.

Several reports have been published indicating high sensitivity of the embryo to the action of blastomogenic agents [1, 8, 9, 11, 13-15]. However, no systematic investigations of transplacental blastomogenesis have yet been carried out.

In earlier studies we examined the transplacental blastomogenic action of urethane on embryonic mouse lung tissue. We found that the affinity of urethane for the lungs, exhibited in experiments on adult animals, is also present when the drug acts via the placenta, for the offspring of these mice developed adenomas of the lungs [6]. Adenomas of the lungs developing from the transplacental effects of urethane also appeared in vitro during experimental organ cultivation of embryonic lung tissue subjected to transplacental action of urethane [4, 5]. The morphogenesis of adenomas developing in vitro and in vivo as a result of the transplacental action of urethane was indistinguishable from the morphogenesis of adenomas developing in experiments on adult mice [2, 3, 12]. In experiments in vitro and in the early stages of postnatal life of mice, in experiments in vivo, a definite relationship between blastomogenic effect and dose of the drug obtained through the placenta was observed. A characteristic feature of adenomas of the lungs developing in vivo and in vitro as a result of the transplacental action of urethane is their extremely rapid development compared with adenomas arising in experiments on adult animals.

In the present investigation we studied the relationship between the blastomogenic effect at different periods of postnatal life of the offspring of the mice and the dose of urethane received via the placenta.

EXPERIMENTAL METHOD

Experiments were carried out on line A female mice (141 animals) which received 10% urethane solution in a dose of 0.2-0.3 ml per injection during the second half of pregnancy. Three doses of urethane were tested: 20-30 mg, as a single injection 1-4 days before parturition (68 mice); 50-60 mg, as two injections 4-8 days before parturition (58 mice); 80-100 mg, as three injections 4-8 days before birth (15 mice). The offspring of the mice were studied at the ages of 2-3, 10-12, and 16-19 weeks. The offspring of animals receiving 80-100 mg urethane in the prenatal period were very weak, and the young animals were often born dead. It was impossible to collect an adequate number of animals of the group, and accordingly only mice aged 2-3 weeks were studied.

The animals were killed with chloroform vapor and their lungs fixed in 10% formalin; after macroscopic examination, histological sections were examined under the microscope.

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TABLE 1. Frequency of Development of Adenomas of the Lungs in Mice at Different Stages of Postnatal Life Following Transplacental Action of Urethane

Dose of urethane	Age of animals (in weeks)								
	2-3			10-12			16-19		
	Number of mice		Incidence of adenomas (in %)	Number of mice		Incidence of adenomas (in %)	Number of mice		Incidence of adenomas (in %)
	Investigated	With adenomas		Investigated	With adenomas		Investigated	With adenomas	
20-30 mg, single injection	46	–	–	15	3	20	7	6	85.7
50-60 mg, two injections	35	2	5.7	15	4	26.6	8	7	87.5
80-100 mg, three injections	15	5	33.3	Not investigated					

EXPERIMENTAL RESULTS

The results obtained are given in Table 1. At macroscopic examination of the lungs of animals aged 2-3 weeks no adenomas were found after all doses of urethane tested. The results of histological examination showed that the transplacental action of urethane in a dose of 20-30 mg led to the development of only preadenomatous changes in the lungs of the mouse progeny: irregular, diffuse, and focal proliferation of the epithelium. No adenomas were found. With a dose of 50-60 mg, besides preadenomatous changes, adenomas developed in two of the 35 animals (5.7%). A dose of urethane of 80-100 mg led to the appearance of adenomas of the lungs in 5 of the 15 mice studies 33.3%). In the remaining animals, just as with smaller doses of the drug, preadenomatous changes were found: diffuse and focal proliferation of the epithelium. Adenomas developing at this period of life of the mice were immature and solid in structure.

A study of the late results of transplacental action of small (20-30 mg) and medium (50-60 mg) doses of urethane revealed adenomas of the lungs of animals aged 10-12 and 16-19 weeks, even when examined with the naked eye. These adenomas were solitary or, more frequently, multiple grayish nodules 0.5-2 mm in diameter, projecting slightly above the surface of the lungs. It was found microscopically that the adenomas were glandular or mixed glandular and papillary in structure, but in their morphological picture they were indistinguishable from adenomas of the lungs developing in experiments on adult animals.

Investigation of the offspring of animals receiving 20-30 mg urethane through the placenta showed that nodules were present on the surface of the lungs in three (20%) of the 15 mice aged 10-12 weeks; the tumors were solitary in all three animals. In 6 (85.7%) of the 7 investigated animals aged 16-17 weeks multiple adenomas of the lungs were found. In 3 mice of one litter the number of nodules on the lungs surface reached 20-22 per lung.

Following transplacental action of 50-60 mg urethane on 15 mice investigated at the age of 10-12 weeks, nodules were found on the surface of the lungs in 4 mice (26.6%), multiple in two cases. Examination of 8 animals aged 16-19 weeks revealed nodules on the surface of the lungs in 7 of them (87.5%): solitary in 2 mice and 2-11 nodules per lung in the rest.

The transplacental action of urethane caused the development of adenomas of the lungs in mice in the early period of postnatal life, by the 2nd-3rd week. In experiments on adult animals, adenomas of the lungs usually develop 6-8 weeks from the beginning of administration of urethane [2, 3, 12]. The frequency of appearance of adenomas of the lungs following transplacental action of urethane, just as in the experiments on adult animals, increased as the duration of the experiment increased (Table 1). It is well known that the blastomogenic effect of a drug (the frequency of tumor development and the latent period of their development) depends on its dose [12]. In our experiments, in the early stages of postnatal life of the animals a definite relationship also was found between the blastomogenic effect and dose of urethane received

in the prenatal period. A similar relationship between blastomogenic effect and dose of urethane when acting via the placenta has also been found in organ cultivation experiments not exceeding 14 days in duration [5].

In the early period of postnatal life of animals receiving different doses of urethane by the transplacental route, a definite relationship was thus found between the blastomogenic effect and dose of the drug. Later, by the 10-19th week, the incidence of adenomas of the lungs became approximately equal in mice receiving small and medium doses of urethane. However, the latent period of development of the tumors differed. As mentioned above, the transplacental action of small doses of urethane did not produce adenomas of the lungs in the early stage of postnatal life of mice. So far as the high incidence of adenomas of the lungs in mice following transplacental action of small doses of urethane is concerned, similar results were obtained by Larsen [14]. In his experiments, following the action of urethane in the late stages of pregnancy the incidence of adenomas in the offspring was much higher than if the same dose acted in earlier periods of pregnancy. In Larsen's opinion this is attributable to the greater blood supply of the fetus and the improved access for urethane in the later stages of pregnancy. Possibly in our experiments also a small dose in the late stages of pregnancy resulted in a high percentage of adenomas of the lungs for the same reasons. High sensitivity of the animals to the blastomogenic action of different doses of ethylnitrosourea acting by the transplacental route has been observed: in the offspring of the rats tumors developed in 100% of cases when the dose used was 10-80 mg/kg [11].

As a result of the transplacental action even of small doses of a blastomogenic substance, tumors can thus arise in the progeny no less frequently than following the action of higher doses.

In this connection the possibility of a blastomogenic action of several therapeutic drugs now being used during pregnancy on a much wider scale must be considered [7]. As experimental results have shown, the embryo is extremely sensitive to the blastomogenic action of even small doses of chemical substances. Such a blastomogenic effect may be difficult to detect at an early age, and this considerably increases the risk because in later life the action of small doses may reach and, in some cases, may surpass the action of larger doses of the drug.

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