EMBRYOTOXIC AND TRANSPLACENTAL ONCOGENIC ACTION OF SYMMETRICAL DIALKYLNITROSAMINES ON THE PROGENY OF RATS

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After administration of a single dose (by mouth) of a series of symmetrical dialkylnitrosamines (nitrosodimethylamine – NDMA, nitrosodiethylamine – NDEA, and nitrosodibutylamine – NDBA) to rats in the maximal doses tolerated by pregnant animals (NDMA, 30 mg/kg; NDEA 200 mg/kg; NDBA 1200 mg/kg), only an increase in the mortality among the embryos was observed on the 3rd, 9th, 10th, and 12th days after fertilization. No teratogenic effect likewise was observed after the intraplacental injection of NDMA and NDEA (100-300 μg into each embryonic sac) on the 13th day of pregnancy. In experiments in which the compounds were given on the 21st day of pregnancy a transplacental oncogenic effect was found in the progeny: NDMA induced tumors in 5 of 20 (25%) animals and NDEA in 15 of 31 (48%) rats that survived until the time of appearance of the first tumor. Malignant tumors were frequently situated in the kidneys.

KEY WORDS: symmetrical dialkylnitrosamines; induction of tumors; mortality of embryos.

Investigation of the principles governing transplacental carcinogenesis may be a promising approach to the study of the pathogenesis of certain tumors found in children [3, 15]. After brief administration in relatively high doses to adult rats, N-nitrosodimethylamine (NDMA) has proved highly effective as an inducer of kidney tumors of various types, including anaplastic tumors and adenosarcomas [5, 11, 12]. The latter are analogous to Wilms' tumor, commonly found in children and regarded as a disembryogenetic neoplasm [2].

It was accordingly decided to study the action of NDMA and also of N-nitrosodiethylamine (NDEA) and N-nitrosodibutylamine (NDBA), belonging to the group of symmetrical dialkylnitrosamines, on rat embryos.

EXPERIMENTAL METHOD

Noninbred albino rats from the Rappolovo Nursery, Academy of Medical Sciences of the USSR, were used. Immediately before use, the substances mentioned were dissolved (NDMA in physiological saline, NDEA and NDBA in sunflower oil) and injected into rats at various times of an accurately dated pregnancy.

In the experiments of series I, in order to detect their embryotoxic and teratogenic action the substances were injected once by means of a gastric tube in the largest doses tolerated by pregnant rats (NDMA, 30 mg/kg, NDEA 200 mg/kg, NDBA 1200 mg/kg), on the 3rd, 9th, 10th, and 12th days of pregnancy. These times were chosen on the basis of the results of previous experiments with NDMA [1], for when given to rats on these days it had an embryotoxic effect. An attempt was made to detect the teratogenic effect of NDMA and NDEA when injected directly into the embryonic sac. For this purpose laparotomy was performed on the rats on the 13th day of pregnancy under ether anesthesia. Both cornua of the uterus were brought out into the wound, after which 0.01-0.02 ml of the NDMA or NDEA solution (100-300 μg) was injected from a fine needle into each embryonic sac from the mesometrial aspect. The control females

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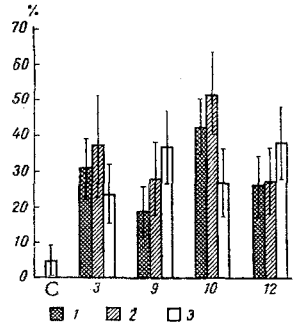

Fig. 1. Comparative embryotoxic activity of symmetrical dialkylnitrosamines for rat embryos: 1) 30 mg/kg NDMA; 2) 200 mg/kg NDEA; 3) 1200 mg/kg NDBA. C) Mortality of rat embryos in control. Abscissa, days of pregnancy at which procedure carried out; ordinate, postimplantation mortality of embryos (in %).

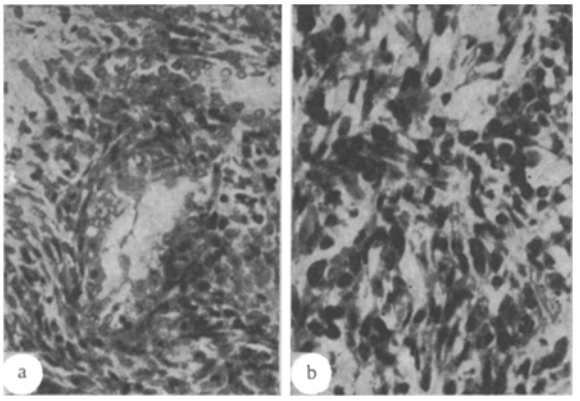

Fig. 2. Adenosarcoma (a) and anaplastic tumor (b) of the kidneys arising in rats after transplacental action of NDEA. Hematoxylin–eosin, magnification: a) 200×, b) 400×.

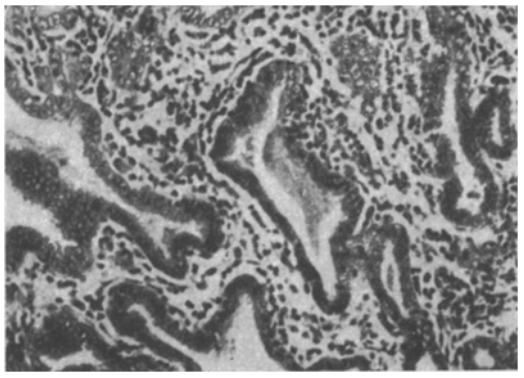

Fig. 3. Cholangioma in a rat after transplacental action of NDEA. Hematoxylin–eosin, 200×.

received an injection of the same volume of physiological saline into the embryonic sac by the same method. The results were read on the 17th-21st day of pregnancy, using the ordinary method of teratologic investigation [1]. In each group of this series of experiments at least six pregnant rats were used.

In the experiments of series II, in order to study the transplacental effect on the progeny, a single injection of NDMA in a dose of 30 mg/kg and NDEA in a dose of 150 mg/kg was given by gastric tube to the rats on the 21st day of pregnancy. The newborn rats were kept together with the mothers during the period of feeding, and were then kept under observation until the time of their natural death. The cadavers of all the animals that died or that were killed when threatened with death were autopsied, and the organs, if suspected of containing tumors, were fixed in Kaiserling's fluid and treated histologically. Material for histological examination was embedded in paraffin wax and sections 5-7 μ in thickness were stained with hematoxylin and eosin and by Van Gieson's method.

EXPERIMENTAL RESULTS

In the experiments of series I, a single injection of each of the compounds into rats on the 3rd, 9th, 10th, or 12th day of pregnancy was followed by an embryotoxic effect only, the intensity of which was approximately the same at all periods of pregnancy studied (Fig. 1). Comparison of the embryotoxicity of the symmetrical dialkylnitrosamines and their toxicity for adult rats [9] shows that in both cases it decreases with lengthening of the alkyl chain.

In the experiments with intraplacental injection of NDMA and NDEA all the embryos died. In a special control series in which the rats received an intraplacental injection of the same volume of physiological saline on the 13th day of pregnancy, the mortality among the embryos (10-12%) was almost indistinguishable from its level in the control animals receiving no treatment whatever (6-7%).

The absence of a teratogenic effect in the experiments with NDMA, NDEA, and NDBA, known to be highly active mutagens [4] and carcinogens [8], indicates that no parallel (or direct link) evidently exists between these manifestations of biological activity.

The results given in this paper agree with the hypothesis expressed earlier [1] that activation of symmetrical dialkylnitrosamines does not take place in the embryo at stages sensitive

to exhibition of the teratogenic effect on account of the absence of dealkylating enzymes. The embryotoxic effect of these substances is probably based on an indirect mechanism mediated through the mother.

In the experiments to study the transplacental oncogenic action of NDMA and NDEA, the first tumor (an adenosarcoma of the kidney) was detected in the progeny 274 days after exposure. By the end of the experiment, in the series receiving NDMA tumors were found in 5 of 20 animals (25%), and in the series with NDEA in 15 of 31 rats surviving until the discovery of the first tumor (48%). Malignant tumors of the kidneys were classified in 7 cases as adenosarcomas (Fig. 2a) and in one case as an anaplastic tumor (Fig. 2b). A small tubular adenoma was found in one rat. Tumors of the liver were found in four rats. In three cases they were cholangiomas (Fig. 3) and in one case a sarcoma, developing from the wall of parasitic cyst. Reticulosis was diagnosed in two rats and a reticulosarcoma of the lung in one. Another four experimental newborn rats had a fibroadenoma of the mammary gland, one had a pituitary adenoma, and one an adenoma of the pale cells of the thyroid gland.

Tumors developed in 9 of the 18 mother rats that survived until the time of appearance of the first tumor (50%). These rats had tumors of the kidneys (hypernephroma in 3 cases, adenosarcoma in 2, adenoma in 3), the ovary (granulosa-cell tumor in 2 cases), reticulosis in 2 cases, and fibroadenoma of the mammary gland in two cases.

Tumors of the kidneys were observed previously in solitary newborn rats (5-7%) after a course of injections of NDMA in the late stages of pregnancy [6], as the present investigation confirmed after a single dose. It was also shown that administration of NDEA leads to a higher frequency of tumor formation in the newborn rats than administration of NDMA. Other workers, in experiments to study the transplacental action of NDEA in rats, found solitary tumors of the kidneys [13], tumors arising in fairly high frequency chiefly in the liver [10], or a broad spectrum of neoplasms [7, 14]. These differences can be explained by differences in the sensitivity of different rats to NDEA.

The possibility of the exhibition of the transplacental oncogenic action of NDMA and NDEA in rats is probably connected both with the significant exposure of the fetus to these substances at the end of pregnancy and with the commencing function of its own enzyme systems activating dialkylnitrosamines in the organs of the fetus.

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