EFFECT OF NEONATAL ANDROGENIZATION ON INDUCTION OF SARCOMAS OF THE UTERUS BY 1,2-DIMETHYLHYDRAZINE IN CBA MICE

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KEY WORDS: 1,2-dimethylhydrazine; testosterone propionate; hyperestrogenization; sarcoma of the uterus.

Neonatal androgenization can cause lasting changes in the endogenous sex hormone level and can thus affect the development of hormone-dependent tumors. Administration of androgen during the first days of life of animals, before sexual differentiation of the hypothalamus is complete, causes long-term disturbances of hypothalamic regulation of gonadotrophic hormones with the establishment of permanent estrus [6, 7]. However, information on the effect of neonatal androgenization on induced carcinogenesis is scanty and is contradictory in nature. For instance, according to some observations [10, 11], neonatal androgenization of rats reduced the frequency of adenocarcinomas of the mammary gland induced by 7,12-dimethylbenz(a)anthracene, whereas according to others [3, 8, 9], it did not affect the development of induced adenocarcinomas but increased the frequency of spontaneous mastopathy and of fibroadenomas of the mammary gland induced by a carcinogen.

1,2-Dimethylhydrazine (DMH) is known to cause the development of sarcomas of the uterus in CBA mice, and administration of sex hormones has a powerful influence on the frequency and latent period of their formation [2, 5]. It was accordingly interesting to study how disturbances of the endogenous sex hormone level caused by neonatal androgenization affect the induction of sarcomas of the uterus by DMH.

EXPERIMENTAL METHOD

During the first days of life female CBA mice (227 animals) received a single subcutaneous injection of 500 μg testosterone propionate in 0.1 ml of an oily solution. After 3 weeks 67 (29.5%) of the androgenized mice were still alive; every week for 20 weeks from the age of 2 months 37 androgenized and 27 intact female CBA mice received DMH in distilled water by subcutaneous injection in a dose of 8 mg/kg body weight. The corresponding control groups consisted of 30 androgenized and 30 intact mice. The time of sexual maturity was identified in the androgenized and intact mice by the time of opening of the vagina. In mice of all four groups the character of the estrous cycles was studied from the picture of vaginal smears taken 1.5 months after the beginning of DMH administration. The animals were killed when palpable tumors were present. All animals which survived were killed 30 days after the beginning of DMH administration. Material fixed in formalin was subjected to the usual histological treatment and sections were stained with hematoxylin and eosin. The numerical results were subjected to statistical analysis by the t and chi-square tests.

EXPERIMENTAL RESULTS

The vagina of the androgenized mice opened earlier than that of the intact animals (25.1 ± 1.3 days compared with 37.6 ± 0.7 days; $P < 0.01$). The study of vaginal smears in mice of the different groups revealed permanent or prolonged estrus in 92.3% of the androgenized mice compared with 15% in the control. Injection of DMH into the androgenized mice did not change the character of their estrous cycles: permanent estrus was observed in 90.6% of mice. Normal cycles were present in only one third of the intact animals receiving DMH, whereas in the remaining mice of this group the cycles were disturbed, with predominance of

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TABLE 1. Effect of Androgenization on Frequency of Detection of DMH-Induced Sarcomas of the Uterus

Experimental conditions	Number of mice surviving until appearance of the first tumor	Time of discovery of tumors, weeks					
		12	15	20	24	26	30
Control	20	0	0	0	0	0	0
DMH	22	0	0	0	0	0	2
Androgenization	21	0	0	0	0	0	0
Androgenization + DMH	30	3	7	18	21	24	27

the estrus phase. In most androgenized females with permanent estrus, development of the interstitial tissue and absence of corpora lutea were observed in the ovaries, and hyperplasia of the epithelium and edema of the stroma were noted in the cervix uteri of these animals.

Tumors of the uterus could be palpated in three androgenized mice receiving DMH, 12 weeks after the beginning of injection of the carcinogen. Toward the end of DMH administration (20 weeks) tumors of the uterus were present in 60%, and after 30 weeks they were present in 90% of androgenized mice receiving the carcinogen (Table 1). All tumors of the uterus were sarcomas, developing from the stroma of the endometrium and (or) myometrium. Stromal polyps of the body of the uterus also were found in five androgenized mice treated with DMH. In intact mice receiving the carcinogen, sarcomas of the uterus developed in two cases (9%), but only after 30 weeks.

Androgenization did not affect the frequency of other DMH-induced tumors. DMH caused the development of intestinal polyps in two intact and in six androgenized mice and hepatoma formation in two intact and one androgenized females. In 11 mice receiving a single dose of testosterone propionate (five of them received DMH), a calculus 0.2-0.5 cm in diameter was formed in the urinary bladder, and in agreement with data in the literature [4], it is suggested that this could be attributed to a disturbance of the hormonal balance and, in particular, to the action of endogenous estrogens.

A single injection of androgen into newborn female CBA mice thus led to a sharp increase in the rate of development and in the frequency of sarcomas of the uterus induced by DMH. We know that DMH lowers the gonadotrophin levels [1] and that estradiol dipropionate shortens the latent period and increases the frequency of DMH-induced sarcomas of the uterus, whereas 17α-progesterone caproate inhibits their development strongly [2]. Injection of the androgen during the first 5 days of life reorganizes hypothalamic function in females toward the male type, after which, with the onset of sexual maturity, the secretion of gonadotropins and, in particular, of FSH, takes place tonically instead of cyclically; under these circumstances permanent estrus is established in the female mice [3, 7, 12]. In the present experiments androgenization of the newborn mice led to earlier sexual maturity than in the control; the permanent estrus observed in most animals was an indicator of a raised endogenous estrogen level. The morphological picture of the cervix uteri (hyperplasia of the epithelium and edema of the stroma) also pointed to hyperestrogenization. These indirect indicators of a raised estrogen level are confirmed by data in the literature showing a sharp rise in the estron and estradiol levels in androgenized rats [7, 8]. The absence of corpora lutea in the ovaries of the androgenized CBA mice indicated lowering of the progesterone level, but according to data in the literature [7, 8], a decrease in the blood gonadotrophin concentration is observed in androgenized animals as early as on the 20th day after birth. Neonatal androgenization thus creates optimal conditions for the development of DMH-induced sarcomas of the uterus (a high estrogen and low progesterone levels). The rise in the endogenous estrogen level induced by neonatal androgenization had the same powerful stimulating effect on induction of sarcomas of the uterus, it will be noted, as administration of exogenous estrogens [2]. However, androgenization by itself (without DMH) did not lead to the development of tumors of the uterus. In precisely the same way, prolonged administration of estradiol dipropionate to intact CBA mice did not induce sarcomas of the uterus in them during 50

weeks of observation [5]. Neonatal androgenization or injection of exogenous estrogens thus has a promotor and (or) cocarcinogenic effect on the development of carcinogen-induced sarcomas of the uterus in mice.

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IMMUNOMORPHOLOGICAL IDENTIFICATION OF MYOEPITHELIAL CELLS IN MIXED MAMMARY GLAND TUMORS IN DOGS

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The histogenesis of the mixed mammary gland tumor is a problem that is still unsolved despite data on its epithelial origin [4, 8]. Discussion of the role and importance of myoepithelial cells (MEC) in the origin and development of the tumor is traditional. There are difficulties in the identification of myoepithelium (ME) at light- and electron-microscopic levels of investigation, due to the morphological and functional variation of the components of the hormonal-dependent mammary gland tissue [2]. With the discovery of contractile proteins (actin, myosin) of smooth-muscle nature in MEC, the way was open for the use of specific immunochemical methods to detect these cells [5, 7]. Because of the structural and functional characteristics of the dog mammary gland, from the technical point of view it affords a convenient object with which to study the difficult problems of histogenesis of mixed tumors of this organ [1].

The object of this investigation was to identify MEC with the aid of monospecific antiserum against smooth-muscle myosin and to study the character of distribution of these cells in mixed tumors of the canine mammary gland.

EXPERIMENTAL METHOD

Materal removed during operations on five dogs, under observation at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, for spontaneous mammary gland tumors was studied. The tumors were: mixed tumor (n = 3), anaplastic carcinoma (n = 1), and adenocarcinoma (n =

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