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Mammary Tumor Induction in Analbuminemic Rats by 7,12-Dimethylbenz(a)anthracene*

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Abstract—*Induction by 7,12-dimethylbenz(a)anthracene of mammary tumors was studied in analbuminemic rats, a mutant strain established from Sprague–Dawley rats which are characterized by the absence of serum albumin and hyperlipidemia. Twenty-three weeks after carcinogen administration, the incidence and average number of mammary tumors and the tumor weight per tumor-bearing rat were respectively* 35.0%, 1.7 ± 0.2 (*S.E.*) *and* 8.9 ± 0.5 g *in analbuminemic rats and* 69.2%, 2.3 ± 0.2 *and* 12.2 ± 2.8 g *in the controls. Associated with this lower mammary tumorigenic response, analbuminemic rats had significantly lower plasma prolactin levels than controls during proestrus at* 7–8 weeks *of age when carcinogen was given* (176 ± 62 *vs* 308 ± 52 ng/ml).

INTRODUCTION

SERUM albumin is a major protein in the plasma, and it is known to be a carrier of many endogenous and exogenous compounds including bile acids, hormones, toxins and probably carcinogens [1].

Recently, we established a mutant strain of analbuminemic rat (NAR) from a stock of SD rats [2]. Although NAR lack albumin, which has so many important functions, they can be maintained ordinarily. They reproduce well under usual conditions of animal care and their only known abnormality is hyperlipidemia [2].

Studies on the function of albumin in carcinogenesis using NAR have shown that these rats show extraordinarily high susceptibility to the induction of bladder cancer by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine [3] but the same susceptibility as controls to liver cancer induced by oral 4-dimethylaminoazobenzene [4].

7,12-Dimethylbenz(a)anthracene (DMBA) is widely used as a potent and selective carcinogen for induction of mammary tumors in rats [5]. As one of a series of experiments on carcinogenesis in NAR, in this work we examined the carcinogenic effects of DMBA in NAR.

The serum level of prolactin, which is a primary hormone for carcinogen-induced mammary tumorigenesis in rats [6], was determined at 7–8 weeks of age when DMBA was administered.

MATERIALS AND METHODS

Animals

NAR and control SD rats bred in the Sasaki Institute were used. Throughout the experiments they were kept 5 to a cage (265 × 425 × 200 mm) with wood shavings and placed on an Iso-rack (Sanki Scientific Co., Tokyo, Japan) in which fresh air filtered by double micro-filters flowed through gently and horizontally from the back wall.

They were maintained in an animal room with air-conditioning (23 ± 2°C, 55 ± 5% relative humidity) and artificial lighting (12 hr of light from 6 a.m. to 6 p.m.) and provided with a commercial diet (CE-2; CLEA Japan Inc., Tokyo, Japan) and tap water *ad libitum*.

Mammary tumorigenesis

DMBA was obtained from Wako Junyaku Co., Tokyo, Japan. Female rats in experimental and

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control groups were each given a single intragastric dose of DMBA (110 mg/kg body wt) dissolved in olive oil at 8 weeks of age and weighed every day after feeding for 5 days. They were then weighed and examined for palpable mammary tumors once a week until week 23 after DMBA administration. Then they were killed and the number and weight of tumors were recorded. Each tumor was fixed in phosphate-buffered formalin and embedded in paraffin, sectioned at 3 μm in thickness and stained with hematoxylin-eosin for histological observation.

The incidences of spontaneous mammary tumors in NAR and SD rats after 2 yr were also compared.

Serum prolactin level

Vaginal smears were taken from 24 rats in each group every morning (9:00–9:30 a.m.) beginning at 5 weeks of age. At 7–8 weeks of age blood was collected from the tail vein under light ether anesthesia on the afternoon (1:00 p.m.) of the 2nd day of diestrus and by decapitation on the evening (5:30 p.m.) of the day of proestrus. Serum prolactin was assayed by radioimmunoassay with a kit from NIH, Bethesda, MO, U.S.A.

Statistics

The significance of the difference in incidences of mammary tumors was evaluated by the χ^2 test and that of serum prolactin levels by Student's t test.

RESULTS

Mammary tumorigenesis

Changes in body weight are shown in Fig. 1. NAR did not differ in appearance from normal SD rats, but they were smaller. The steady increases in body weight of the young rats was interrupted for 2 or 3 days after DMBA administration, but growth recommenced thereafter.

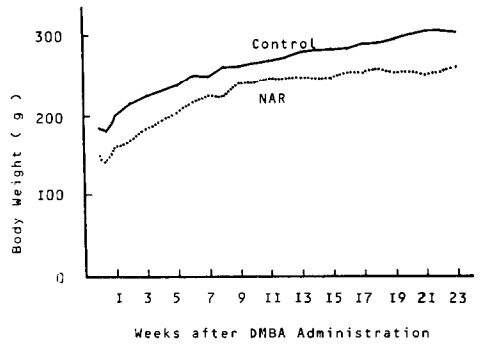

Fig. 1. Changes in body weight in control rats and NAR.

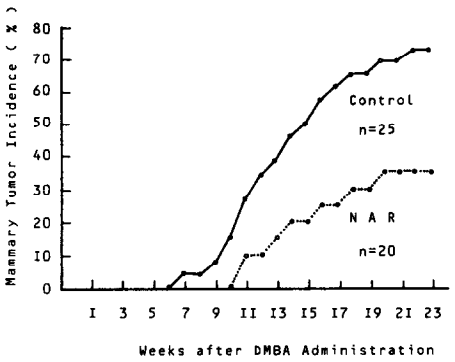

Fig. 2. Changes in cumulative mammary tumor incidence in control rats and NAR.

Table 1. General features of mammary tumorigenesis in NAR and control rats 23 weeks after DMBA administration

	NAR	Control
Incidence (%)	35.0 (7/20*)	69.2 (18/25)
Latent period (weeks)	14.7 ± 1.3†	13.6 ± 1.0
Number‡	1.7 ± 0.2	2.3 ± 0.2
Weight‡ (g)	8.9 ± 0.5	12.1 ± 2.8
Histological type		
Adenoma		1
Fibroadenoma		5
Fibroma		1
Intraductal papilloma	2	
Papillotubular carcinoma		3
Medullary tubular carcinoma	5	8
Tubular carcinoma	2	5
Papillary adenocarcinoma		1
Adenocanthoma	2	1

*Number of rats with tumors/total number of rats examined.
†Mean ± S.E.
‡Number means number of tumors per tumor-bearing rat, and weight is the weight per tumor.

The cumulative incidence of mammary tumors in each group is shown in Fig. 2. The incidence in NAR was significantly lower than that in the controls 15 weeks after DMBA administration ($P<0.05$). As shown in Table 1, the average number and weight of tumors at the end of the experiment were also lower in NAR than in the controls, although the differences were not statistically significant. The latent periods were 13.6 ± 1.0 weeks in SD and 14.7 ± 1.3 weeks in NAR.

Histological findings on mammary tumors are also summarized in Table 1. Benign tumors were found in 2 of 11 NAR and 7 of 25 SD rats, the other tumors being malignant. The differences in the incidences of these types of tumors in the two groups were not significant.

The incidence of spontaneous mammary tumors was much lower in NAR than in the controls (Fig. 3).

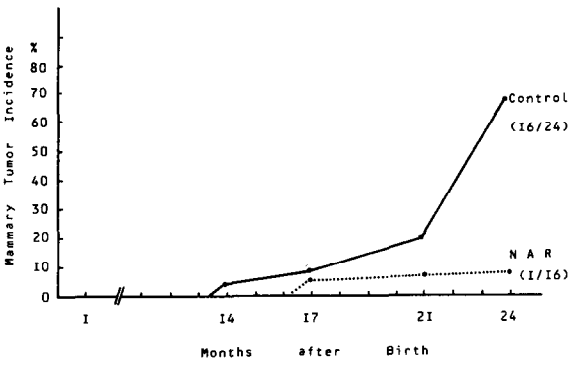

Fig. 3. Spontaneous mammary tumor incidence in control rats and NAR.

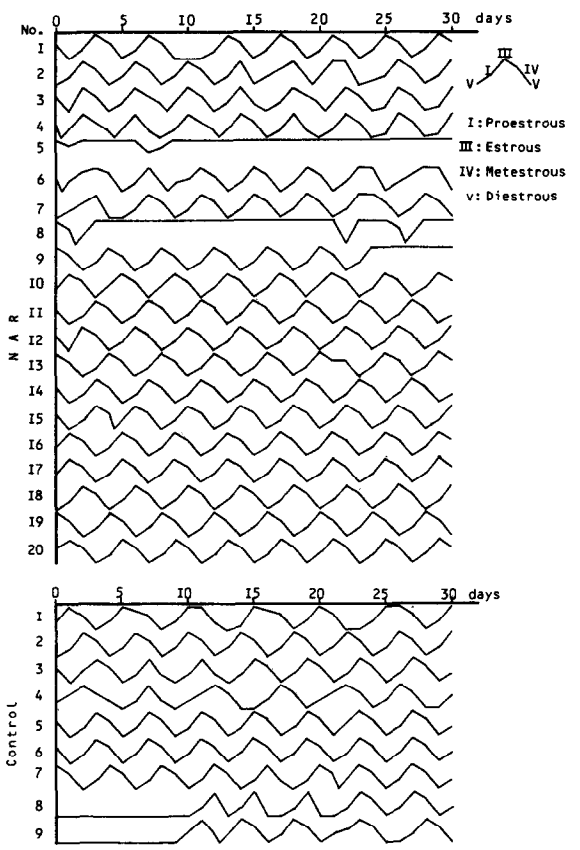

Fig. 4. Representative examples of the pattern of estrous cycles in control rats and NAR.

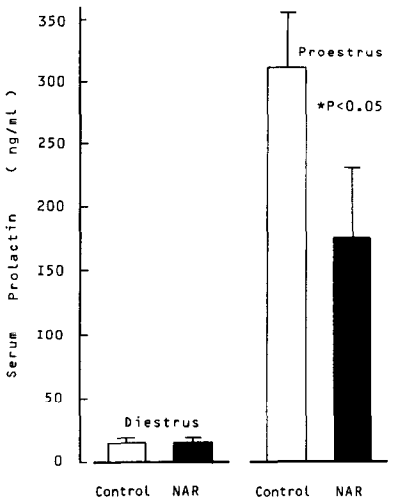

Fig. 5. Serum prolactin level at 7–8 weeks of age in control rats and NAR. NAR: Nagase analbuminemia rat.

Serum prolactin level

Representative examples of the patterns of estrous cycles in NAR and SD rats are shown in Fig. 4. Little difference was seen between the two strains. The proestrous serum prolactin levels at 7–8 weeks of age were significantly lower in NAR than in the controls, but the diestrous prolactin levels in the two groups were the same (Fig. 5).

DISCUSSION

This study shows that mammary tumorigenesis induced by DMBA was much lower in NAR than in the controls.

Prolactin is the primary hormone for experimental mammary tumorigenesis. Besides promoting progression of malignant foci, it also enhances mammary tumor development by increasing mitotic activity to make conditions favorable for the action of carcinogenic agents [7].

In this study the prolactin level at 7–8 weeks of age, when DMBA was administered, was significantly lower in NAR than in the controls. In SD rats suppression by CB-154 (ergocryptine) of the serum prolactin level at the time when DMBA was administered resulted in marked inhibition of DMBA-induced mammary tumorigenesis [8]. Thus the lower mammary tumor response to DMBA in NAR could mostly be ascribed to the lower circulating prolactin level at the critical time for mammary tumor development.

However, other factors such as lipid metabolism or weight gain seem to affect mammary tumorigenesis. As reported [2, 9], NAR are deficient in serum albumin and are hyperlipidemic. The relation between fat and cancer has been the subject of experimental studies for years, and evidence from epidemiological and experimental studies suggests that dietary fat is a factor in breast cancer risk. Thus from the viewpoint of lipid metabolism, a rather high incidence of mammary tumor might be expected in NAR.

It has been clearly demonstrated that under- or overnutrition has a significant effect on mammary tumor development. As shown in Fig. 1, the body weight of NAR was about 40 g less than that of SD

rats. But changes of body weight in both strains not treated with DMBA were similar to those of the experimental group. Moreover, the external appearance of NAR is not greatly different from that of normal rats, except that NAR are smaller and, moreover, NAR show no significant pathological abnormalities.

It is possible that some abnormal metabolism of DMBA in NAR contributes to the low induction of mammary tumors in these rats. However, our observation that NAR also show a much lower incidence of spontaneous mammary tumors, for which prolactin is also essential [6], supports the idea that a low level of prolactin is mainly responsible for the low induction of mammary tumors by DMBA, in NAR. Similar results have also been obtained in normal rats: temporary suppression of pituitary prolactin secretion in young normal rats resulted in marked inhibition of spontaneous mammary tumorigenesis later in life [10].

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