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Response to Adreno-Ovariectomy and/or Pituitary Grafting of Carcinogen-Induced Mammary Tumors in Rats with Different Growth Potential*

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Abstract—*Growth response to hormone manipulation was studied in 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors in rats with different growth potential. The rats were divided into two groups according to the growth rates of the first mammary tumors; the rapidly and the slowly growing groups which had tumors of more than 100% and less than 20% increase in size during 3 weeks after appearance, respectively. In the rapidly growing group, the tumor growth was stimulated by the single pituitary grafting in intact hosts, but the treatment had no effect in adreno-ovariectomized hosts. By contrast, in the slowly growing group, the pituitary grafting prevented the adreno-ovariectomy-induced regression of tumors, however, the tumor growth of intact hosts was not affected by the grafting. The results indicate the distinct difference in growth response to mammotropins between the rapidly and the slowly growing mammary tumors induced by DMBA. In the rapidly growing group, about 70% and 30% of mammary tumors were progressive and static, respectively, and those in the slowly growing group were 7% and 89%, respectively, indicating that growth potential of this type of mammary tumor is host specific rather than the tumor specific. There was no relationship between normal and neoplastic mammary tissues in response to hormonal manipulation.*

INTRODUCTION

MAMMARY tumors in rats induced by 7,12-dimethylbenz[a]anthracene (DMBA) are highly hormone responsive and widely used as one of the representative animal models for human breast cancer. However, it is well known that there are large variations in the growth potential among individual tumors. Moreover, tumor response to hormones may vary with the stage of development [1, 2] and established tumors may undergo spontaneous regression, independently of the prevailing hormonal state [3]. Since the results of most tumor experiments are expressed in terms of average tumor response, the response of the individual tumors is often lost in this process and this may sometimes cause confusing or incorrect conclusions.

In this paper, we studied the effects of adreno-ovariectomy and/or pituitary grafting on the rapidly and the slowly growing DMBA-induced mammary tumors in rats as a possible step to evaluate their values for an animal model.

MATERIALS AND METHODS

Female Sprague–Dawley rats received the single i.v. injections of 5 mg fat emulsion of DMBA (The Upjohn Co., Kalamazoo, Michigan, U.S.A.) at 50 days of age and checked for palpable mammary tumors every 7 days throughout the experiment beginning 3 weeks after DMBA injection. The number of tumors and their sizes expressed in terms of the geometric mean of the major two diameters were recorded. The tumor-bearing rats

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were divided into two groups according to the percentage changes in the size of the first tumors during the 3 weeks after appearance; tumors which increase in size more than 100% into the 'rapidly growing group' and tumors which increase in size less than 20% into the 'slowly growing group'. Each group was further divided into the following four subgroups at 3 and 6 weeks after the first tumor appearance in the rapidly and the slowly growing groups, respectively. The 1st subgroup remained intact and served as the control; the 2nd subgroup was grafted with a single anterior pituitary each under the kidney capsule, the 3rd subgroup was bilaterally adreno-ovariectomized and the 4th subgroup was adreno-ovariectomized and simultaneously grafted with pituitary.

Tumor-bearing rats were assigned to individual subgroups on a random basis as they became available and the resultant latency period of tumors, time lapse between DMBA administration and occurrence of palpable mammary tumors, was calculated.

Besides the first mammary tumors, the percentage changes in size during the 2 weeks after appearance of the other tumors which appeared before treatment were also recorded in each rat. Category of changes was defined as follows; progressive, $>50\%$ increase; static, $<50\%$ change, and regressed, $>50\%$ decrease in the tumor size. The latency period of tumors were also calculated.

At 4 and 7 weeks after treatment in the rapidly and the slowly growing groups, respectively, experimental rats were killed by decapitation. Instant control rats in both groups were killed in the evening of proestrus or diestrus. Blood was collected from the trunk and the third thoracic mammary glands were used for the wholemount preparations.

Development of normal mammary end-bud system was rated from 1 to 7 in increments of 1 [4].

Serum prolactin level was determined by radioimmunoassay using the kit donated by NIAMDD, NIH, U.S.A.

Significance of difference was evaluated by the Duncan's multiple range test or χ^2-test.

RESULTS

The growth curve of mammary tumors in each group which appeared first is shown in Fig. 1. In the rapidly growing group, the pattern of growth of mammary tumors before treatment was not different among subgroups. While the growth of tumors retarded after 4 weeks of appearance in the intact control rats, the tumors of the intact rats continued to grow linearly after the pituitary grafting. However, the drastic decline in tumor size after adreno-ovariectomy was not prevented by the simultaneous pituitary grafting.

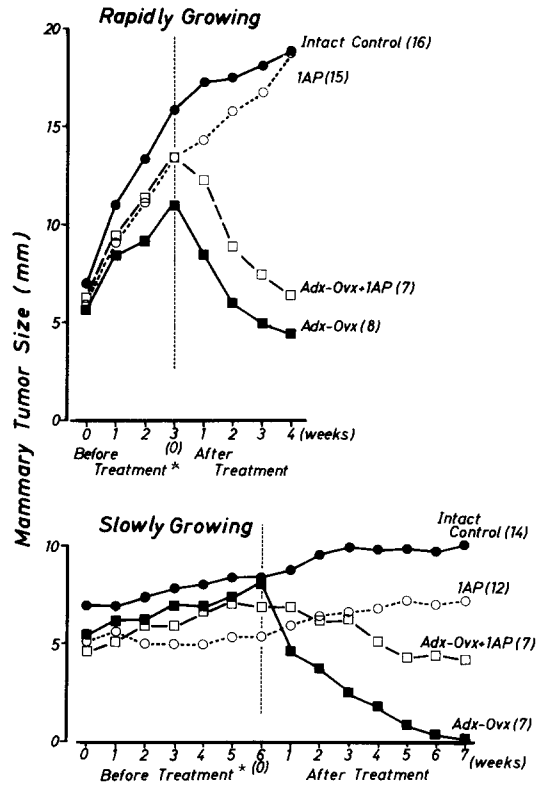

*Fig. 1. Effects of adreno-ovariectomy (adx-ovx) and/or a single pituitary grafting (1AP) on the growth of DMBA-induced mammary tumors in rats which appeared first and were different in growth potential. Number of tumors examined is indicated in the parentheses. *See Table 1 for details of treatment. (----) Adreno-ovariectomy and/or a single pituitary grafting.*

The case was quite the contrary in the slowly growing group. The single pituitary grafting showed no influence on the growth of mammary tumors in the intact animals. Meanwhile, the pituitary grafting significantly prevented tumor regression by adreno-ovariectomy.

The degree of adreno-ovariectomy-induced regression was similar in both the rapidly and the slowly growing tumors.

All the results were well reflected by the percentage changes in the tumor size before and after treatment in each subgroup (Table 1). In either the rapidly or the slowly growing group, there was no significant difference among subgroups in the percentage changes of the sizes of the first tumors before treatment, while some differences were observed in the latency periods of tumors betweeen subgroups in both groups.

Table 1. Effects of adreno-ovariectomy (adx-ovx) and/or a single pituitary grafting (1AP) on the percentage changes in DMBA-induced mammary tumors in rats which appeared first and were different in growth potential (means ± S.E.M.)

Group and treatment*	No. of tumors examined	Latency period† (weeks)	Percentage changes in mammary tumor size		
			Before treatment	After treatment	
				4 weeks	7 weeks
Rapidly growing					
Intact control	16	11.0±1.1	144±19	23±9	—
1AP	15	14.8±1.0	117±10	56±11‡	—
Adx-ovx	8	8.4±0.3	102±7	−61±9	—
Adx-ovx+1AP	7	9.2±0.4	142±20	−55±4	—
Slowly growing					
Intact control	14	14.0±1.1	21±6	15±10	27±14
1AP	12	13.3±0.8	24±7	25±19	44±25
Adx-ovx	7	10.9±0.5	49±17	−76±12	−98±3
Adx-ovx+1AP	7	9.4±0.5	34±7	−30±8§	−52±16§

*Percentage changes in the sizes of mammary tumors which appeared first were more than 100% and less than 20% during 3 weeks after appearance in the rapidly and the slowly growing groups, respectively. Rats were bilaterally adreno-ovariectomized (adx-ovx) and/or grafted with a single pituitary each under the kidney capsule (1AP) at 3 and 6 weeks after the first tumor appearance and mammary tumor size was checked every 7 days for 4 and 7 weeks after treatment in the rapidly and slowly growing groups, respectively.

†Time lapse between DMBA-administration and occurrence of palpable mammary tumors.

‡Differs from intact control at $P<0.05$.

§Differs from adx-ovx control at $P<0.01$.

After treatment, in the rapidly growing group, the percentage change in mammary tumor size was significantly higher in the intact rats with the single pituitary grafts than in the control, whereas there was no difference in the changes between adreno-ovariectomized rats and adreno-ovariectomized, pituitary grafted rats. On the contrary, in the slowly growing group, the percentage change of mammary tumor size after treatment was significantly higher in adreno-ovariectomized, pituitary grafted rats than in adreno-ovariectomized rats at both 4 and 7 weeks after treatment, but no difference was seen between intact controls and the intact, pituitary grafted rats.

The rate of decline of tumor size after adreno-ovariectomy was similar in the rapidly and the slowly growing groups at 4 weeks ($-61\pm9\%$ vs $-76\pm12\%$).

As shown in Table 2, in the rapidly growing group, approximately 70 and 30% of mammary tumors which appeared before treatment were progressive and static, respectively, and none was regressed.

In the slowly growing group, the percentages of progressive, static and regressed mammary tumors were 7, 89 and 4%, respectively.

No difference in the latency period was observed between mammary tumors in each category in both groups.

Normal mammary glands of intact control rats consisted of fine, well branched duct system only or ducts with small end-buds, being rated from 1 to 3 in both the rapidly and slowly growing groups (Fig. 2). The single pituitary grafting resulted in the conspicuous proliferation of end-bud system, the degree of which became marked with the advance of time after grafting (4.3 ± 0.3 in the rapidly growing group at 4 weeks vs 5.3 ± 0.4 in the slowly growing group at 7 weeks).

While no difference in the number of branching of ducts was observed between the intact controls and the adreno-ovariectomized rats, the end-bud system disappeared and ducts became slender by adreno-ovariectomy in either the rapidly or the slowly growing group. The regression was prevented a little by the pituitary grafting in both groups.

Serum prolactin levels in each group at autopsy are shown in Fig. 3. There were little differences in the levels between the corresponding subgroups of the rapidly and the slowly growing groups. Prolactin levels of the intact control rats were extremely high in the

Table 2. Differences between groups in the number and percentage of DMBA-induced mammary tumors in each category before treatment

Group and treatment*	Number of tumors examined	Progressive† (%)	Static (%)	Regressed (%)
Rapidly growing				
Intact control	27	63 (17)‡	37 (10)	0
1AP	22	73 (16)	27 (6)	0
Adx-ovx	11	73 (8)	27 (3)	0
Adx-ovx + 1AP	8	75 (6)	25 (2)	0
Mean		71	29	0
Latency period (weeks)§		11.9 ± 0.6	11.4 ± 0.6	—
Slowly growing				
Intact control	26	12 (3)	88 (23)	0
1AP	19	0	89 (17)	11 (2)
Adx-ovx	28	11 (3)	89 (25)	0
Adx-ovx + 1AP	16	6 (1)	88 (14)	6 (1)
Mean		7	89	4
Latency period (weeks)		13.2 ± 0.8	12.2 ± 0.5	10.7 ± 1.5

*See Table 1 for details of treatment.
†Progressive, >50% increase; static, <50% change, and regressed, >50% decrease in size during 2 weeks after appearance before treatment.
‡Number of mammary tumors belonging to the category.
§Time lapse between DMBA administration and occurrence of palpable mammary tumors.

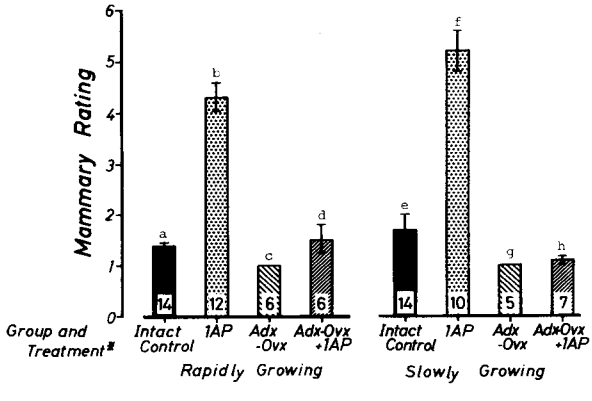

*Fig. 2. Effects of adreno-ovariectomy (adx-ovx) and/or a single pituitary grafting (1AP) on development of normal mammary end-bud system in rats bearing DMBA-induced mammary tumors with different growth potential (means ± S.E.M.). Number of rats examined is indicated in each column. *See Table 1 for details of treatment. b/a, c, d; a/c; f/e, g, h; e/g: P<0.01. c/d: P<0.05.*

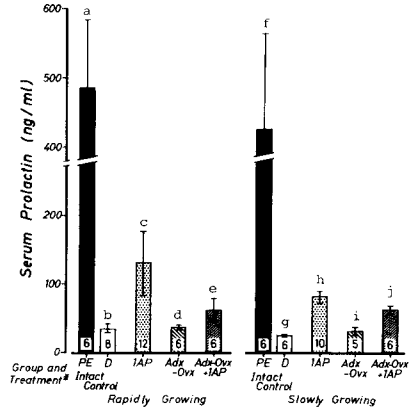

*Fig. 3. Effects of adreno-ovariectomy (adx-ovx) and/or a single pituitary grafting (1AP) on serum prolactin levels at autopsy in rats bearing DMBA-induced mammary tumors with different growth potential (means ± S.E.M.). Number of samples is indicated in each column. *See Table 1 for details of treatment. PE: proestrus. D: diestrus. a/b, c, d, e; f/g, h, i, j; h/g, i; j/g, i: P<0.01. c/b, d: P<0.05.*

evening of proestrus and were low at diestrus comparable to the levels of adreno-ovariectomized rats. The levels of intact animals with pituitary grafts were elevated significantly when compared to the intact control at diestrus, the adreno-ovariectomized rats and adreno-ovariectomized, pituitary grafted rats, although the difference from the adreno-ovariectomized, pituitary grafted rats was not statistically significant. The adreno-ovariectomized, pituitary grafted rats were also higher than the adreno-ovariectomized rats and the intact rats at diestrus in the level, while the differences were statistically significant only in the slowly growing group.

DISCUSSION

In the present study, DMBA-induced mammary tumors in rats could be classified as rapid and slow growing. The growth of the rapidly growing mammary tumors, but not slowly growing tumors was stimulated significantly in intact animals by the single pituitary grafting. On the contrary, adreno-ovariectomy induced regression of mammary tumors was significantly prevented by the single pituitary grafting only in the slowly growing tumors, but not in the rapidly growing tumors, despite the fact that the rate of regression after adreno-ovariectomy was similar in these two types of mammary tumors. Little difference in serum prolactin levels at autopsy was seen between the corresponding subgroups of the rapidly and the slowly growing groups. All findings indicate that responsiveness of DMBA-induced mammary tumors to prolactin is altered by the conditions of other hormones, especially adrenal and ovarian steroids. Rapidly growing tumors are more sensitive to prolactin than slowly growing tumors under the presence of adrenals and ovaries. Meanwhile, under the absence of these organs, rapidly growing tumors are less sensitive to prolactin. The causes of this different responsiveness are not understood at present. It is generally accepted that the binding to the specific membrane or cytoplasmic receptor sites of the cells is one of the initial and essential steps to manifest hormone action. Estrogen and prolactin influence their receptors each other in DMBA-induced mammary tumors in rats [5–7]. In estrogen target tissues, progesterone receptor is largely dependent upon estrogen [8]. Moreover, it has recently been reported that adrenoglucocorticoid can modulate prolactin receptor in normal mammary glands of mice [9, 10]. Therefore, the dependence of response to the pituitary grafting upon adrenals and ovaries in the rapidly growing and the slowly growing tumors obtained in the present study may partly be ascribed to the differences in the effects of hormone manipulation on the receptors to mammotropic hormones between these two types of mammary tumors. In any case, it should be in mind to use these mammary tumors with different growth potential separately according to the experimental purposes.

In each subgroup of the rapidly growing group, approximately 60–70% of tumors which appeared before treatment were progressive and none was regressed. On the other hand, about 90% of tumors were static in each subgroup of the slowly growing group. These suggest that the growth potential of DMBA-induced mammary tumors is specific to the individual host rat rather than a characteristic of the individual tumor.

The view that there is the difference in responsiveness to prolactin between normal and neoplastic mammary tissues [11] is supported by the present findings; while marked difference was seen in the response to pituitary grafting between the rapidly and the slowly growing tumors, there was no difference in the development of normal mammary glands between the host rats bearing mammary tumors with different growth potential.

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