DMBA-induced Mammary Carcinogenesis, in Relation to Rebound Increase of Serum Prolactin after Suppression with Bromocryptine in Pubescent Virgin Rats

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Summary

Twenty six, 31 and 53 young female Sprague-Dawley rats in Groups A, C and E, respectively, were administered 10 mg/Kg Bromocryptine daily by intubation for 3 weeks from 31 to 51 days of age. Twenty five rats each in Groups B and D served as the controls. Tail blood was collected for radioimmunoassay of prolactin from only meta- and di-estrus rats in Groups A, B and E around 10 am at the age of 51-58 days, and the sampling was not repeated in any animal. A rebound increase of serum prolactin level occurred within 3 days after the termination of Bromocryptine treatment. A single dose of 5mg/rat DMBA in Groups A and B and 2.5mg/rat in Groups C and D was given orally around 11 am at the age of 52 days, and the carcinogenesis with DMBA was evaluated 22 weeks after the single administration of carcinogen. Mammary adenocarcinoma developed in some animals, but no other tumors could be found. When a single weaker dose of DMBA was administered orally on the day after the cessation of the 3 week treatment with Bromocryptine, the chemical induction of mammary carcinogenesis was enhanced distinctly.

Introduction

Almost all Sprague-Dawley female rats go into estrus and exhibit cyclic surges of serum prolactin (PRL) between 35-42 days of age¹⁾. Suppression of PRL secretion with 2-Bromo- α -ergocryptine-methanesulfonate (Bromocryptine), which did not interfere with other endocrine processes, occurred in adult female rats at nontoxic dose levels²⁾. Spontaneous or chemically induced mammary tumors have been reported to be inhibited by chronic suppression of PRL secretion in young female rodents²⁻⁷⁾. In this communication, the influence of a 3-week treatment with Bromocryptine was investigated in pubescent virgin rats, when a single dose of 7,12-dimethylbenz(α)anthracene (DMBA) was given on the day after the cessation of

Bromocryptine treatment.

Materials and Methods

Sprague-Dawley female rats were purchased at the age of 23 days from Japan Clea Lab., Osaka, and kept 4 to 5 rats per polycarbonate cage with wooden shavings on an Isolack. The animal room was air-conditioned and artificially illuminated (14 hr of light; 6 am-8 pm). The animals were provided a commercial diet and tap water freely. At the age of 30 days, they were divided into the following experimental groups; 26 rats in Group A, 25 in Group B, 31 in Group C, 25 in Group D, and 53 in Group E. Daily intubation of 10mg/Kg of Bromocryptine (Parodel of Sandoz Ltd, Basel), suspended in 0.5ml of 2.5% arabian gum solution, was carried out with a gastric tube in Groups A, C and E for 3 weeks from 31 to 51 days of age. The control rats (Groups B and D) received daily the arabian gum solution containing no Bromocryptine. Vaginal examinations of all rats were done every day early in the morning at the age of 35 to 68 days. Peripheral blood was collected from the tail veins of some meta- and di-estrus animals in Groups A, B and E around 10 am at the age of 51 to 58 days, and the sampling was not repeated in any animal. The blood sampling was done without anesthesia as quickly and gently as possible. Sera of several proestrus and estrus rats in Group B were also collected to evaluate the radioimmunoassay technique used for PRL. All of the sera were frozen and kept at -20°C until assayed. Serum PRL levels were measured by the radioimmunoassay method of Kato et al.⁸⁾, which were performed kindly by Dr. Kato, Shimane Medical University.

A single dose of 5mg/rat DMBA in Groups A and B and 2.5mg/rat in Groups C and D was given orally with a stomach tube around 11 am at the age of 52 day. DMBA (Eastman Chemical Co., Rochester) was purified by Florisil chromatography and recrystallized from acetone-ethanol, and each dose of refined DMBA was dissolved in 1 ml of sesame oil. As the control, some rats in Group E were given 1 ml of sesame oil containing no DMBA at the age of 52 days. All of the experimental animals were palpated twice a week to detect mammary tumors for 4 months from 6 to 22 weeks after DMBA administration. At the age of 29 weeks (5 months after DMBA administration), all rats were killed by decapitation, and the tumors produced were examined histopathologically.

Results

Vaginal openings were found in all animals at the age of 36-40 days, and these averages were similar in all groups. A regular sex cycle was observed in Bromocryptine-treated rats as well as in untreated controls, although a few rats given 5mg/rat DMBA revealed a delayed estrus cycle. At the age of 52 days (day of DMBA administration), meta- and di-estrus signs

(M-D) in vaginal smears were observed in 12 rats in Group A, 12 in Group B, 17 in Group C, and 15 in Group D, respectively. In Group E, M-D were observed in 10 rats at the age of 51 days, in 11 at the age of 53 days, in 12 at the age of 54 days, and in 9 at the age of 55 days. The serum PRL level was distinctly low in some meta- and di-estrus rats of Group E just after the last intubation of Bromocryptine. It was higher in some-meta and di-estrus Bromocryptine-treated Group A rats than in Bromocryptine-untreated Group B rats at the age of 52 days (just before the single administration of DMBA). At a similar age, 3 proestrus rats of Group B had a serum PRL level of 180, 176 and 136 ng/ml, and 3 estrus rats in Group B had a level of 51, 83 and 91 ng/ml, respectively. The average serum PRL level in the meta- and di-estrus rats of Group E was significantly high 2-3 days after the termination of Bromocryptine treatment, compared with the untreated control Group B, as shown in **Table 1**. The rebound elevation of serum PRL levels lasted for 3 days and peaked 2 days after the cessation of a 3 week treatment with Bromocryptine in pubescent virgins, although the sex cycle was regular in almost all the animals.

The average body weight did not differ significantly from each other DMBA-treated groups, as indicated in **Table 2**. At the age of 52 days (day of DMBA administration), 17 tumor-developed rats in Group A exhibited proestrus and estrus signs (P-E) in 10 smears and M-D in 7 smears, and 15 tumor developed rats in Group C had P-E in 9 smears and M-D in 6 smears. Therefore, the development of mammary adenocarcinomas in Bromocryptine-treated rats was not significantly related to the stage in the sex cycle at the time of DMBA administration. In Bromocryptine-untreated rats, however, the induction rates of mammary adenocarcinomas were, respectively, 9/13 (69%) in the P-E subgroup of Group B, 4/12 (33%) in the M-D subgroup of Group B, 4/10 (40%) in the P-E subgroup of Group D, and 1/15 (7%) in the M-D subgroup of Group D. The incidence of cancer 5 months after DMBA

Table 1 A Rebound Increase of Serum Prolactin in Pubescent Virgin Rats after Cessation of A 3 Week Treatment by Bromocryptine

Group	Age (days)	Days after cessation of 3 weeks' treatment	Number	Serum prolactin ^o (ng/ml)
1	52	untreated control	12	33.0±4.03
2	51	0	10	2.1±0.29**
3	52	1	12	70.8±30.51*
4	53	2	11	96.6±9.82**
5	54	3	12	66.8±42.19*
6	55	4	9	46.9±30.19
7	56-58	5-7	11	23.0±10.30

^o; Mean±standard deviation. Statistically significant difference (*p<5%, **p<1%) from the untreated control group.

Table 2 Effect of Rebound Prolactin Secretion after Bromocryptine Treatment on Mammary Carcinogenesis by Single Dose of DMBA in Pubescent Rats

Experimental group	Oral dose of 5.0mg/rat DMBA		Oral dose of 2.5mg/rat DMBA	
	Bromocryptine -treated	Untreated control	Bromocryptine -treated	Untreated control
Number of animals	26	25	31	25
Body weight (g) [°]				
DMBA-feeding (Age of 52 days)	182± 7.8	189± 5.5	180±12.6	187±11.1
Autopsy (Age of 206 days)	283±23.6	278±21.4	293±33.3	286±20.9
Mammary tumors				
Incidence	17/26	13/25	15/31	5/25
Induction rate (%)	65.5	52.0	48.3	20.0
Number of tumors/ a tumor-bearing rat [°]	2.2±0.9	2.2±0.8	1.9± 0.9	1.2±0.4

[°] ; Mean±standard deviation.

All of the mammary tumors detected were certified histopathologically to be adenocarcinomas, and no other tumors were detected at autopsy.

administration tended to be higher in the rats fed a single dose of DMBA at proestrus or estrus stage than in rats fed at metaestrus or diestrus stage. As indicated in **Table 2**, the index of mammary carcinogenesis in Group A was not significantly different from that in Group B, but that in Group C was 2 times higher than that in Group D. The enhancement with PRL could be detected more distinctly in the weaker dose group.

All tumors detected at autopsy were diagnosed as mammary adenocarcinomas, and no other tumors including benign mammary tumors could be detected at 7 months of age, when survivals of Group E had no tumors.

Discussion

Chronic suppression of PRL secretion with Bromocryptine has been reported to inhibit the development of mammary tumors²⁻⁷⁾. Cohen⁶⁾ described that the rats treated with Bromocryptine at the age of 35 to 50 days exhibited decreased tumor incidence, a longer latency period, and fewer tumors per animal, when 5mg/rat DMBA was administered orally at the age of 50 days, compared to the vehicle controls. Sylvester et al.⁷⁾ described that Bromocryptine treatment beginning 1 day before and for 7 days after an intravenous injection of 5mg/rat DMBA decreased all parameters of mammary tumor development 16 weeks after DMBA administration, as compared with the untreated controls. A variety of experimental studies have shown that increased PRL levels can have dimetrically opposite effects on the development of mammary tumors including cancer, depending on the time of PRL enhancement⁴⁾. As a

rebound effect, elevation of serum PRL level occurred within 3 days after the last intubation of Bromocryptine in meta- and di-estrus rats. Therefore, almost all animals in Group A and C would have higher PRL levels in serum at the age of 52-54 days than the animals in Groups B and D, especially non-estrus ones. When a single weaker dose of DMBA was given on the day after the cessation of a 3 week treatment with Bromocryptine (higher PRL stage), the chemical induction of mammary carcinoma was enhanced more distinctly. To obtain a high rate (100%) and rapid (3 months) production of DMBA-induced mammary cancer, either a single massive dose (130mg/kg) or twice large doses (68mg/kg) within 4-day interval was given orally to the Sprague-Dawley virgins in the 8th week of age⁹⁾. The parameters of the mammary tumorigenesis with a weaker dose (weak initiator) might be influenced more by many factors of the hosts (promotor or inhibitor)¹⁰⁾. In the present experiment, the mammary carcinogenesis with an oral dose of 13.5mg/kg was enhanced significantly by the rebound increase of PRL, compared with that with 27mg/kg of DMBA. Nagasawa et al.¹¹⁾ stated that the percentage of progressive mammary tumors, tumor growth rate, and both number and wet weight of tumors per tumor-bearing animal were higher in the rats injected with 5mg of DMBA in proestrus than in the rats injected in diestrus. In Bromocryptine-untreated groups, the parameter of mammary carcinogenesis was higher in the rats given a single dose of DMBA at the proestrus and estrus stages than in the rats given the dose at the metaestrus and diestrus rats. This effect was also more distinct in the weaker carcinogen group. However, Bromocryptine-treated groups did not exhibit the effect of sex cycle at the time of DMBA administration. Therefore, PRL surge might introduce the above-mentioned effect of sex cycle.

Nagasawa et al.¹¹⁾ reported that DNA synthesis in the mammary gland of virgin Sprague-Dawley rats at the age of 50 days was significantly higher on the day of proestrus, when PRL had a surge, than on the day of diestrus. Higher DNA synthesis in the mammary gland, which may be evoked by a rebound elevation of serum PRL, during the time when DMBA is active might be one of the most important enhancers in the carcinogenesis with a smaller amount of carcinogen. Moreover, sensitivity of the mammary gland to carcinogen could not be denied judging from the level of its morphological differentiation¹²⁾. Bromocryptine probably synchronizes the mammary gland cell through the suppression of PRL secretion, and the synchronized cell population may become more susceptible to the carcinogen in 1-6 days after the termination of Bromocryptine treatment. Mammary gland development from birth to adulthood has been described in rats by several authors, and some authors expressed it by counting the density of histological units of the mammary parenchym per unit area. Recently, Sakaki¹⁵⁾ has described the cell kinetics with BrdU-labeling index of distinctly classified structural units of the mammary parenchym in paraffine sections of the largest part of the 4th

mammary gland of Sprague-Dawley virgin rats. She stated that the glandular cell division was diminished in the differentiation-retarded mammary parenchym with Bromocryptine, and that morphological development of the mammary gland was significantly high 1-4 days after the peak of rebound elevation of PRL after Bromocryptine treatment. Finally, she concluded that mammary gland DNA synthesis may be mostly controlled by PRL. The enhancement of mammary carcinogenesis with DMBA given the day after a 3 week treatment of Bromocryptine in puberty would be introduced by active DNA synthesis the day after the peak of rebound elevation of PRL.

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