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Promoting action of prolactin released from a grafted transplantable pituitary tumor (MtT/F84) on rat prostate carcinogenesis

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Summary

The potential modifying effects of high prolactinemia on rat prostate carcinogenesis was investigated. Male F344 rats were treated at 5 times of 5-week intervals with s.c. injections of 3,2'-dimethyl-4-aminobiphenyl (DMAB), each injection following 3 weeks pretreatment with dietary ethinyl estradiol. After completion of the carcinogen administration stage, rats received multiple s.c. transplantations of a prolactin producing transplantable pituitary tumor, MtT/F84 until sacrifice at week 51. The effects of additional or single treatment with bromocriptine, a prolactin suppressing agent, were also investigated. The body, liver and kidney but prostate weights were significantly increased in the groups given MtT/F84. Although the development of prostate carcinomas was not affected by the observed hyperprolactinemia, the incidences of atypical hyperplasia of both ventral and lateral prostate were significantly enhanced. The findings thus indicate that prolactin may have promoting potential for prostate carcinogenesis.

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Keywords: prostate; carcinogenesis; prolactin; rat; 3,2'-dimethyl-4-aminobiphenyl.

Introduction

Prolactin has been shown to play important roles in regulation of the growth and function of the prostate gland. For example, in the presence of testosterone, it acts as a growth-promoting factor for both the dorsolateral [11] and ventral lobes [5,12,19] of the prostate in the rat. This synergism between the two hormones appears to occur mainly during sexual maturation. The presence of prolactin receptors in the rat prostate has been demonstrated in several investigations [1,3,10,18,21]. Witorsch et al. [20] revealed that hyperplastic or dysplastic epithelial cells and adenocarcinoma cells retain prolactin receptors in contrast to atrophic epithelial cells which lose their binding activity. Despite the fact that high levels of prolactin can inhibit the growth of normal prostate [13] and transplantable prostate carcinoma cells [14]. Investigation of latent carcinomas in human prostate tissue revealed elevated prolactin levels, suggesting possible promotional effects of this hormone on tumor growth [22].

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In the present study the effects of prolactin released from a subcutaneously inoculated transplantable pituitary tumor [7] on prostate carcinogenesis were investigated in 3,2′-dimethyl-4-aminobiphenyl-initiated rats [9,15,16].

Materials and methods

Male F344 rats (purchased from Charles River Japan, Inc., Kanagawa, Japan), 6 weeks old and weighed approximately 124 g at the beginning of the experiments, were housed in plastic cages on hard wood chip-bedding in an air-conditioned room with a 12h-12h light-dark cycle and given food (Oriental MF; Oriental Yeast Co., Ltd., Tokyo) and water ad libitum. Ethinyl estradiol (EE) was purchased from Sigma Chemical Co., St. Louis, MO. U.S.A. DMAB was obtained from Matsugaki Pharmaceutical Co., Osaka. The cyclic dietary regimen with EE-containing diet and basal diet was essentially the same as that described in previous papers [15,16], i.e., EE diet for 3 weeks and basal diet for 2 weeks, repeatedly. The animals were divided into 7 groups (25 rats each for Groups 1 to 3 and 20 for Groups 4 to 7). The cyclic dietary regimen was applied to the animals of all groups and repeated 5 times. The rats in Groups 1 to 4 were given a single s.c. injection of DMAB at 50 mg/kg body weight 2 days after each change to the basal diet.

From experimental week 25, the animals were treated as follows; MtT/F84 transplantation for Groups 1 and 5, MtT/F84 transplantation plus bromocriptine (CB154, Sandoz Ltd., Basel, Switzerland) for Groups 2 and 6 and CB154 alone for Groups 3 and 7. Group 4 served as a non-treatment control given neither the tumor cells nor CB154. CB154 was mixed with powdered diet at a concentration of 3.75 mg/kg diet and was administered orally ad libitum. All surviving animals were killed at week 51 and all major organs and tissues including macroscopically observable lesions were processed for routine histological examination.

Transplantation of MtT/F84

Tumor cell preparations for transplantation were made following the methods described in a previous paper [7]. Tumor tissue, which had been maintained in the subcutis of female F344 rats, was minced in MEM solution and gently shaken in the same medium with 300 units of collagenase/ml (Worthington Biochemical Co., NJ, U.S.A.) at 37°C. After washing off the enzyme and debris with MEM, tumor cell viability was examined by the trypan blue exclusion test. Viable tumor cell suspensions were mixed with equal amounts of 50% brain homogenate, obtained separately from syngeneic rats and homogenized in MEM. Mixture aliquots (0.06 ml) of this tumor cells and brain homogenate were inoculated into both inguinal regions with different numbers of cells; 1×10^5 cells for the right side and 1×10^3 for the left side. Because of estrogen-dependency of this tumor cells, 17β-estradiol was added to the cell mixture at a concentration of 0.1 μg/0.01 ml. A pellet of 17β-estradiol (E_2 pellet) was also inserted under the subcutis between the scapula for the first 1 week. These surgical procedures were performed under anesthesia with ethyl ether. In all of the MtT/F84 inoculated sites, tumors become visible and palpable after about 3 weeks with 1×10^5 tumor cells and about 5 weeks with 1×10^3 cells. Tumor(s) were removed when they grew to a maximum size of 2 cm or when the surface became ulcerated. All of the first tumors were removed by week 32 and the 2nd ones were removed by week 37. After removal of both tumors, transplantation was repeated at week 39 with the same procedures as described above for week 25. After the 2nd transplantation, tumors at the sites receiving fewer inoculated cells persisted until the end of the experiment.

Serum prolactin levels

Blood samples were collected from the cervical vein of 3 rats in each group at week 32 (7 weeks after the 1st transplantation) under ethyl ether light anesthesia and from the aorta of all

surviving rats at the end of the experiment, sera being stored at −20°C until assay. Serum prolactin levels were measured as described previously with NADDK rat prolactin kit reagents [8].

Results

Animal growth

Insertion of E_2 pellets under the subcutis at the time of the initial transplantation of the MtT/F84 tumor cells retarded growth of the rats and they were therefore removed after 1 week. Growth recovered thereafter. Hyperplastic mammary glands with multiple small milk cysts were noticed at each time of operation, suggesting elevation of serum prolactin levels. Many animals which died or were killed upon becoming moribund later than week 40 were found to have MtT/F84 metastatic lesions in several organs. This was the main reason why the experiment was terminated at week 51.

Table 1 summarizes the average body, liver, kidney and prostate weights at the end of the experiment. MtT/F84 transplantation significantly increased the average body, liver and kidney weights, regardless of whether with or without DMAB treatment; increased rates were about 11% for body, 230% for the liver and 170% for the kidney. These increased values were suppressed by co-treatment with CB154 but this was statistically significant only in the liver case in animals pretreated with DMAB. In the group without DMAB pretreatment, all the 3 weights were significantly decreased by CB154. The weights of the prostate (ventral prostate) and associated tissues, however, were not affected by either MtT/F84 or CB154.

Serum levels of prolactin

At week 32, slight elevation in the levels of prolactin was associated with the transplantation of MtT/F84 (Table 2). CB154 alone appeared to slightly depress prolactin level. The effects of MtT/F84 and CB154 on prolactin were much clearer at week 51; group 1 with MtT/F84 transplantation showed 4-fold higher values than did group 4, CB154 exerting marked suppressive effects.

Neoplastic and preneoplastic lesions in the prostate and seminal vesicles

Lesions arising in the prostate including the seminal vesicles were classified into atypical

Table 1. Final body and organ weights.

Groups	Average weights (g)					
	No. of rats	Body	Liver	Kidney[a]	Prostate	
					Ventral	Other[b]
1. DMAB, Mt[c]	7	419.6**	20.3***	1.70***	0.36	1.78
2. DMAB, Mt, CB154	12	404.9*	16.1***	1.45***	0.38	1.82
3. DMAB, CB154	23	351.8*	8.2	0.98	0.34	1.61
4. DMAB	15	377.7	8.7	1.01	0.34	1.60
5. Mt	9	436.8	18.6	1.88	0.40	1.89
6. Mt, CB154	11	411.5	14.2	1.42	0.34	1.68
7. CB154	20	372.1	8.4	1.04	0.35	1.77

[a] Only right kidney data presented.

[b] Including lateral and dorsal prostate, coagulating gland and seminal vesicles.

[c] MtT/F84 tumor.

Significantly different from group 4 values at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Table 2. Serum prolactin levels.

Groups	Average level of serum prolactin ($\times 10^3$ ng/ml)	
	Week 32	Week 51
1. DMAB, Mt	1.29 ± 0.73 (3)[a,b]	1.15 ± 1.11 (6)**
2. DMAB, Mt, CB154	1.12 ± 0.36 (3)	0.97 ± 0.72 (12)**
3. DMAB, CB154	0.64 ± 0.21 (3)	0.11 ± 0.06 (21)**
4. DMAB	0.84 ± 0.27 (3)	0.27 ± 0.18 (15)
5. Mt	NE[c]	1.11 ± 1.05 (9)*
6. Mt, CB154	NE	0.28 ± 0.25 (10)
7. CB154	NE	0.07 ± 0.04 (20)

[a] Mean ± S.D.
[b] No. in parentheses = no. of animals examined.
[c] Not examined.
* Significantly different from the group value 6 at $P < 0.05$.
** Significantly different from the control value (group 4) at $P < 0.01$.

hyperplasias and carcinomas (Table 3). Animals which lived longer than 50 experimental weeks were included in the effective numbers. Carcinomas were found only in the ventral prostate, and demonstrated the same histological pattern as reported previously [9,15,16]. However, their incidences were very low; 1/9 (11%), 1/14 (7%), 0/23 and 3/15 (20%) in groups 1—4, respectively. No carcinomas were present in the groups not given DMAB. The incidence of atypical hyperplasias of ventral prostate in Group 1 (8/9, 89%) was significantly higher than the carcinogen alone control value (7/15, 47%), but that of Group 3 (4/23, 17%) was lower. The incidence of atypical hyperplasias in the lateral prostate in Group I (4/9, 44%) was also significantly higher than the control value (1/15, 7%).

There were no inter-group differences in the incidences of atypical hyperplasias of the seminal vesicles.

The dorsal prostate of rats receiving MtT/

Table 3. Incidences (%) of atypical hyperplasias and carcinomas of the prostate and seminal vesicles.

Groups	Effective no. of rats[a]	Prostate			Seminal vesicles
		Ventral		Lateral	
		Atypical hyperplasia	Carcinoma	Atypical hyperplasia	Atypical hyperplasia
1. DMAB, Mt	9	8 (89)***	1 (11)	4 (44)*	4 (44)
2. DMAB, Mt, CB154	14	9 (64)	1 (7)	5 (36)	6 (43)
3. DMAB, CB154	23	4 (17)	0	1 (4)	7 (30)
4. DMAB	15	7 (47)	3 (20)	1 (7)	9 (60)
5. Mt	10	2 (20)	0	0	0
6. Mt, CB154	12	0	0	0	0
7. CB154	20	0	0	0	0

[a] Effective numbers include all rats which survived for longer than 50 weeks.
Significantly different from the control values (group 4), at $^*P < 0.05$ and $^{***}P < 0.001$.

Table 4. Incidences of tumors in organs other than the accessory sex organs.

Groups	Effective no. of rats[a]	Large intestine	Preputial gland	Pancreas	Subcutis/skin	Liver	Kidney	Peritoneum
1. DMAB, Mt	9	0	2(22)	8(89)	0	0	0	0(11)
2. DMAB, Mt, CB154	14	1(7)	3(21)	13(93)	1(7)	0	1(7)	0
3. DMAB, CB154	23	2(9)	2(9)	14(61)	1(4)	0	0	1(4)
4. DMAB	15	3(20)	2(13)	11(73)	0	1(7)	0	0
5. Mt	10	10	0	1(10)	0	0	0	0
6. Mt, CB154	12	0	2(17)	1(8)	0	0	0	0
7. CB154	20	0	0	0	0	0	0	0

[a]Effective numbers include all rats which survived for longer than 50 weeks.

F84 transplantation exhibited epithelial enlargement and increased exfoliation of epithelial cells into the acinar lumen. No observable non-neoplastic alterations related to MtT/F84 were evident in other parts of the accessory sex organ complex.

Neoplastic lesions in organs other than the prostate

Table 4 summarizes the incidences of neoplasia in organs other than the accessory sex organs. Primary tumors were found in the large intestine (adenoma/adenocarcinoma), preputial gland (adenoma/adenocarcinoma), skin/subcutis (fibroma and sebaceous adenoma), pancreas (acinar cell focus and nodule), liver (hyperplastic nodule), kidney (renal cell adenoma) and peritoneum(mesothelioma). There were no significant inter-group differences in the incidences of these tumors.

Metastatic lesions of MtT/F84 were evident in 4 organs, the lung and adrenal glands being found to be involved at more than 58% incidence (Table 5). Other lesions considered to be related to the presence of MtT/F84 tumor were cell hypertrophy and vacuolization of hepatocytes in the liver and marked tubular dilatation with eosinophilic casts in the kidney. These lesions were observed in all rats which received MtT/F84 tumor transplantation but in none of the animals without the transplantation. No obvious morphological alterations were evident in the pituitary glands.

Discussion

In the present work transplantation of MtT/F84 was found to significantly increase the serum levels of prolactin, treatment with CB154 being associated with a slight suppression. Although the variation between individual animals was relatively large, functional effectiveness of the transplantation of MtT/F84 and treatment with CB154 was demonstrated by the fact of hyperplasia observed in the mammary glands and by the differences

Table 5. Metastatic lesions from the MtT/F84 transplanted tumor.

Groups	Effective No. of rats	No. (%) of animals with metastasis in			
		Lung	Kidney	Liver	Adrenal glands
1	9	8(89)	3(33)	0	6(67)
2	14	13(93)	2(14)	0	12(86)
5	10	8(80)	3(30)	2(20)	7(70)
6	12	7(58)	1(8)	0	8(67)

noted in the body, liver and kidney weights. The data for DMAB initiated lesion development showed that high levels of prolactin enhanced the appearance of atypical hyperplasias in the ventral and lateral prostate, this effect being reduced by lowering of prolactin with CB154. Although no effects of prolactin were observed on the development of prostate carcinomas, atypical hyperplasias are accepted as precancerous lesions [9,15,16,20], and thus the findings indicate that prolactin can exert promoting potential on prostate carcinogenesis. This is in accordance with the reports that dysplastic epithelial cells of the ventral prostate of ACI/seg rats retain prolactin receptors [20] and that prolactin acts as a growth-promoting factor for rat prostate [5,11,12,19].

In our previous experiment, a cyclic dietary administration of EE followed by administration of DMAB was demonstrated to be very effective for induction of a high incidence of prostate carcinomas in rats [9,15]. In the present investigation, this same method was utilized but the cycle was repeated only 5 times instead of 10 to shorten the duration of treatment with carcinogen. Presumably the smaller total dose of DMAB and shorter experimental period (51 weeks) might explain why the incidence of prostate carcinomas was very low.

With regard to the mechanism underlying prolactin effects, Barañoa [2] reported that this hormone elevates activity of steroid 5α-reductase in the prostate epithelium, leading to growth-promotion of prostate epithelium in the presence of testosterone. A recent investigation also revealed that MtT/F84 may secrete growth hormone [6] and the presently observed changes in body, liver and kidney weights in animals under the influence of transplanted MtT/F84 may be attributed to this factor. The lack of any effect of prolactin on the appearance of atypical hyperplasia in the seminal vesicles and tumor development in non-sex organs is of interest for further investigation in terms of organ specific mechanism. It is necessary to investigate using a way by which only prolactin level is elevated, such as the transplant of the pituitary gland in the spleen [4] or under the kidney capsule [3,17], for example.

The present experiment revealed that MtT/F84 to be highly metastatic. Repeated operation for removal of tumors might have exerted strong effects on any remnants and thus account for the high metastatic rates. This tumor might thus provide a good animal model for investigation of metastasis.

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