Inhibition of a Transplantable Pancreatic Carcinoma by Castration and Estradiol Administration in Rats¹

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ABSTRACT

Influence of sex steroids on the growth of an azaserine-induced transplantable rat pancreatic carcinoma, DSL-2, was studied. This established transplantable tumor has been maintained in syngeneic rats. Inbred male Lewis rats were pretreated with castration and s.c. implantation of 1.0-mg 17β-estradiol (CAS: 50-28-2; estradiol) pellets at 7 weeks of age. Tumor cells were inoculated s.c. on the back of intact male, castrated male, or 17β-estradiol-treated castrated male rats. Additional male rats served as non-tumor-bearing controls. There was no difference in the body weight between tumor-bearing and non-tumor-bearing male rats. A distinct difference in the tumor growth was observed in variously conditioned recipients. In castrated male hosts, the serum testosterone levels and the epididymis weights were significantly decreased, and the tumor weights were significantly less as compared to intact control hosts. Additional pretreatment with 17β-estradiol caused a markedly slower growth of tumors and increases of the serum 17β-estradiol levels and the pituitary weights in castrated male recipients. The remarkable response of tumor growth to castration was also observed in a fast-growing tumor derived from DSL-2. Moreover, close positive relationships between tumor weights and the activities of both serum amylase and lipase were observed. Results showed that the pretreatment with castration alone or in combination with 17β-estradiol treatment was able to inhibit the growth of the transplantable tumor. In addition, tumor cells had an ability to produce amylase and lipase, and the amount of enzymatic activity was related to the tumor volume. Thus, these data indicate that the transplantable rat pancreatic carcinoma retains physiological function. Our previous study has shown the modulation by sex steroids of azaserine-induced preneoplastic lesions of pancreas in rats. Therefore, androgens and estrogens may play key roles as promoters and inhibitors during the process of pancreatic carcinogenesis.

INTRODUCTION

Pancreatic cancer is one of the most difficult neoplastic diseases in regard to early diagnosis and treatment. Its age-adjusted incidence has been higher among men than among women (1, 2). Following a report in 1981 (3) showing the presence of estrogen receptors in human pancreatic carcinoma, interest in an association between pancreatic cancer and sex hormones has been increasing (4–7). We have recently shown in a short-term model that treatments with both castration and estrogen were highly effective in inhibiting the development and growth of azaserine-induced preneoplastic lesions of the rat pancreas (8). Results indicated that sex hormones modulate pancreatic carcinogenesis during the early stage. In general, pancreatic carcinogens are considered to initiate a sequence of focal cellular proliferative changes, some of which progress to the formation of grossly visible tumors (9, 10). Foci of atypical acinar cells are a good indicator for the early stage of pancreatic carcinogenesis and a predictor for malignant neoplasms in rats, although not all foci progress into the advanced stage. It is unknown whether malignant cells that acquired autonomy still have such a hormone responsiveness. Thus, the present study was carried out to clarify this point by using a transplantable rat pancreatic carcinoma, DSL-2. In addition to the measurement of 17β-estradiol and testosterone levels, pituitary glands were evaluated as a biological marker of 17β-estradiol action, since estrogens stimulate prolactin secretion (11–14).

The DSL-2 carcinoma usually kills syngeneic hosts within 2 months when grafted s.c. on the back. While tumor cells show anaplasia, their ability to produce exocrine enzymes has been reported (15, 16). Therefore, it was also investigated whether malignant cells still retained physiological function characteristic of acinar cells and whether their enzymic production is related to the tumor growth. Amylase and lipase activities in the sera of rats bearing tumors were measured and used as an enzymatic marker for tumor cell growth and function.

MATERIALS AND METHODS

Animals. Inbred male Lewis rats (Charles River Breeding Laboratories, Wilmington, MA) were housed in a temperature (21 ± 1°C) and light (12-h light and 12-h dark cycle)-controlled room. They were fed standard rat chow ad libitum and were given deionized water. Rats were weighed and palped for tumors weekly.

Tumor. The transplantable carcinoma of pancreas, DSL-2, was established and has been maintained in our laboratory by serial transplantation in syngeneic rats and in cryopreservation since 1977. This tumor was originally induced in a male Lewis rat by azaserine treatment and was histologically diagnosed as pancreatic acinar cell carcinoma (8). At the seventh transplant generation, the dissociation of tumor cells was carried out for transplantation. The tumor, s.c. grafted in syngeneic male rats by serial cell transplantation, usually became palpable within 3 weeks.

The procedure for cell suspension was as follows. Fresh tumor tissue was removed, washed in Ca²⁺-Mg²⁺-free Hanks’ balanced salt solution (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum, and cut into small pieces. These small blocks were minced and filtered through 100-mesh/inch stainless steel mesh, by rinsing with an ice-cold Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Dissociated tumor cells were counted by hemocytometer and prepared 5 to 10 × 10⁶ cells/ml of cell suspension. Viability was determined by the trypan blue dye exclusion test. All procedures were performed at 4°C under sterile conditions. Tumor cells (1.2, 1.6, or 2.2 × 10⁶) were inoculated s.c. on the back of a syngeneic hosts. DSL-2 (the seventh and tenth transplant generations) and a fast-growing tumor, a subline derived from DSL-2 at the fifth transplant generation (DSL-2-F), were used.

Castration and Estradiol Treatments. As the pretreatment, castration, or estrogen pretreatment was performed at 7 weeks of age under somonpentyl anesthesia (sodium pentobarbital; The Butler Co., Columbus, OH). Estradiol pellets containing 1.0 mg 17β-estradiol (CAS: 50-28; estradiol) designed for 60-day timed release (Innovative Research of America, Toledo, OH) were implanted s.c. on the back of host rats.

Histological Study. All rats were autopsied at a single interval after transplantation in each experiment. The tumor, epididymis, pituitary gland, pancreas, and mammary gland were removed, weighed, and fixed in Bouin’s solution. Paraffin sections were routinely stained with hematoxylin & eosin and examined histologically.

Steroid Assay. Blood samples were drawn from the axillary vein of all rats at autopsy under light ether anesthesia. Sera were immediately

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Effects of pretreatment with castration and 17β-estradiol on the growth of the transplantable pancreatic carcinoma were studied in Experiment 1. The body weight and the Ab W$^3$ and Re W (mg/100 g body weight) of epididymis and pituitary gland in tumor-bearing rats conditioned variously and non-tumor-bearing male rats are summarized in Table 1. The mean body weight of tumor-bearing intact male rats (group 1) was not different from that of non-tumor-bearing intact male rats (group 4). In the castrated recipients (groups 2 and 3), both mean Ab and Re W of epididymis were significantly decreased ($P < 0.05$) as compared to intact males (group 1). The serum testosterone level was sharply reduced ($P < 0.05$) in the castrated groups 2 and 3 (Fig. 1). On the other hand, an elevation of serum 17β-estradiol levels in castrated male recipients receiving 17β-estradiol pretreatment (group 3, $P < 0.05$) was observed. Also the mean Ab and Re W of the pituitary glands in castrated male recipients was significantly increased ($P < 0.05$) when 17β-estradiol was given.

**Tumor Growth.** A distinct difference in the tumor growth was observed in variously conditioned hosts. The tumor grew much faster in intact males than in castrated males with or without 17β-estradiol pretreatment. As shown in Fig. 2, the Ab W of tumors were 1.52 ± 0.37, 0.84 ± 0.14, and 0.18 ± 0.04 in the intact, castrated, and castrated plus 17β-estradiol-pretreated groups, respectively. The Re W of tumors were 511 ± 132, 310 ± 38, and 85 ± 19 mg in these groups. Although statistical significance was not detectable between castrated male and intact male hosts, the pretreatment by castration combined with 17β-estradiol significantly inhibited tumor growth ($P < 0.05$).

In addition, there was a close relationship between tumor weights and the serum levels of either testosterone ($P = 0.0027$) or 17β-estradiol ($P = 0.0013$). The tumors were smaller in castrated groups 2 and 3 with the lower testosterone levels and 17β-estradiol-pretreated group 3 with the higher 17β-estradiol levels. Thus, the tumor growth was affected by altered host hormonal condition following the pretreatment with castration or 17β-estradiol.

**Serum Amylase and Lipase Activities.** Enzymic productivity of tumor cells was demonstrated by measuring the serum amylase and lipase activities. Mean values of amylase and lipase in tumor-bearing rats were the highest in intact males, moderate in castrated males, and lowest in castrated males receiving 17β-estradiol (Table 2). In non-tumor-bearing intact males these were $922 ± 37$ IU/dl and $1.0 ± 0.6$ Sigma-Tietz units, respectively. There were significant differences ($P < 0.05$) in the activity of both serum amylase and lipase between 17β-estradiol-treated castrated male hosts (group 3) and intact male hosts (group 1). Moreover, the amylase activity declined further in castrated male hosts when the pretreatment with 17β-estradiol was added ($P < 0.05$). The pattern in the enzymic activity in tumor-bearing hosts was similar to that found in the tumor growth.

Figs. 3 and 4 show a correlation between tumor weights and the activity of serum amylase or lipase at autopsy. Both amylase and lipase values were linearly elevated, corresponding to an increase in the tumor weights: amylase, $r = 0.95$, $P < 0.001$; lipase, $r = 0.81$, $P < 0.001$. Thus, these findings showed that the transplantable pancreatic carcinoma had an ability to produce amylase and lipase, and the productivity was closely related to the tumor volume.

Tumors grew in the transplanted site and were encapsulated. Macroscopically, the tumor mass was vascular and dark-red. There was no metastasis to other organs. Microscopically, tumors showed diffuse proliferation with a solid arrangement and focal cystic change (Fig. 5). Fewer mitotic figures in tumor

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Body (g)</th>
<th>Ab</th>
<th>Re$^c$</th>
<th>Epididymis (mg)</th>
<th>Pancreas (mg)</th>
<th>Ab</th>
<th>Re$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>304 ± 6</td>
<td>7.2 ± 0.7</td>
<td>2.4 ± 0.2</td>
<td>141.0 ± 7</td>
<td>46.5 ± 2.0</td>
<td>970 ± 43</td>
<td>319 ± 10</td>
</tr>
<tr>
<td>Cast$^a$</td>
<td>267 ± 6</td>
<td>8.8 ± 1.1</td>
<td>3.3 ± 0.4</td>
<td>33.5 ± 2.0$^b$</td>
<td>12.5 ± 0.7$^b$</td>
<td>803 ± 22</td>
<td>300 ± 5</td>
</tr>
<tr>
<td>Cast + 17β-estradiol$^c$</td>
<td>212 ± 2</td>
<td>21.5 ± 1.3$^d$</td>
<td>10.1 ± 0.5$^d$</td>
<td>37.3 ± 3.4$^d$</td>
<td>17.7 ± 1.7$^d$</td>
<td>810 ± 39</td>
<td>382 ± 20$^d$</td>
</tr>
<tr>
<td>Intact$^d$</td>
<td>316 ± 6</td>
<td>9.5 ± 0.3</td>
<td>2.9 ± 0.2</td>
<td>165.0 ± 9</td>
<td>52.0 ± 2.2</td>
<td>962 ± 25</td>
<td>305 ± 9</td>
</tr>
</tbody>
</table>

* Tumor cells (1.6 × 10$^6$) were inoculated s.c. on the back of syngeneic rats 7 days after the pretreatment and rats were autopsied 21 days later.
* Mean ± SE of six rats per group.
* Relative (mg/100 g body weight).
* Host rats were castrated at 7 weeks of age.
* Significantly different from group 1.
$^c$ Relative (mg/100 g body weight).
$^d$ Non-tumor-bearing intact male rats.

The abbreviations used are: Ab W, absolute weight(s); Re W, relative weight(s).
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Fig. 1. Tumor weights of hosts pretreated with castration alone or in combination with 17β-estradiol. *, P < 0.05; Intact, intact male; Cast, castrated; E2, estradiol; columns, mean; bars, SE.

Fig. 2. Serum levels of testosterone and estradiol in tumor-bearing hosts conditioned variably. *, P < 0.05; Intact, intact male; Cast, castrated; E2, estradiol; T, testosterone; columns, mean; bars, SD.

Table 2 Serum amylase and lipase activities in tumor-bearing rats pretreated with castration alone or in combination with 17β-estradiol

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>No. of rats</th>
<th>Amylase (IU/dl)</th>
<th>Lipase (STU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>6</td>
<td>1313.0 ± 182</td>
<td>10.8 ± 3.2</td>
</tr>
<tr>
<td>Cast</td>
<td>6</td>
<td>952.0 ± 77</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>Cast + E2</td>
<td>6</td>
<td>649.0 ± 20*</td>
<td>1.5 ± 0.3*</td>
</tr>
</tbody>
</table>

* Tumor cells (1.6 x 10⁶) were inoculated s.c. on the back of syngeneic rats 7 days after the pretreatment and rats were autopsied 21 days later.
* Mean ± SE.
* Host rats were castrated at 7 weeks of age.
* Pellets containing 1.0 mg 17β-estradiol were given s.c. on the back of rats.
* Significantly different from group 1.
* Significantly different from group 2.

Fig. 3. Relationship between tumor weights and the serum amylase activity in all groups. Correlation coefficient; r = 0.95, P < 0.001.

Fig. 4. Relationship between tumor weights and the serum lipase activity in all groups. Correlation coefficient; r = 0.81, P < 0.001.

Table 3. There was a significant difference in Ab W between intact and castrated male hosts bearing DSL-2 (P = 0.027). Similarly, this distinct response to castration was also observed in the fast growing tumor, DSL-2-F: both Ab W (P = 0.027) and Re W (P = 0.032) were significantly less in castrated hosts as compared with intact male hosts. The tumor growth was markedly inhibited by the pretreatment with castration. Moreover, even the tumor with a higher growth rate was profoundly affected in its growth by the alteration in the host hormonal condition following castration.

DISCUSSION

In the present study, we investigated whether pretreatment with castration and 17β-estradiol is able to inhibit the growth of transplantable rat pancreatic carcinoma. A marked inhibition of the tumor growth induced by combined pretreatment with castration and 17β-estradiol was shown in Experiment 1. Both the Ab and Re W of tumors was reduced by approximately 85% in 17β-estradiol-treated and castrated male hosts, as compared to intact male controls. Although pretreatment with castration alone caused a 40% decrease in the tumor growth, a statistical significance was not detectable in Experiment 1. However, the distinct response of tumor growth to castration was shown in Experiments 2 and 3. There were significant differences in the tumor weights between intact male and castrated male hosts.
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Fig. 5. Upper, histological appearance of pancreatic carcinoma (7th generation of DSL-2) transplanted in a male Lewis rat, showing anaplasia. Note cancer nests surrounded by thick connective tissue and lymphocytic cell infiltration. H & E; bar, 200 μm. Middle, higher magnification of upper section, showing diffuse infiltration of tumor cells into connective tissue and several mitoses. H & E; bar, 100 μm. Lower, appearance of same tumor shown in upper section, showing poorly differentiated pancreatic carcinoma with solid pattern. Higher incidence of mitotic figures can be seen. H & E; bar, 50 μm.

Fig. 6. Histological appearance of pancreatic carcinoma transplanted in 17β-estradiol-treated and castrated male Lewis rat. Pleomorphism of nucleus in size and tumor cells with the polarity, basal nucleus and cytoplasm granules in the apical portion, can be seen. The large space (*) is interstitial with fluid accumulation. H & E; bar, 50 μm.

bearing tumors. Thus, the pretreatment with castration alone or in combination with 17β-estradiol was highly effective in the inhibition of the growth of rat transplantable pancreatic carcinoma. Interestingly, even in the fast-growing tumor, growth inhibition by more than 50% was induced by castration. This striking effect was somewhat unexpected because it seemed likely to be more difficult to inhibit the growth of such a tumor. This suggests that even highly malignant cells may be responsive to an altered host hormonal milieu, such as a depletion of androgen.

Serum testosterone levels in tumor-bearing male hosts were sharply reduced with castration, while additional pretreatment with 17β-estradiol induced an elevation of serum 17β-estradiol levels. Positive relationships between the weights of pituitary or epididymis and the pretreatment with 17β-estradiol or castration were clearly observed. In general, implantation of estrogen pellets induces an increase in the circulating prolactin levels and pituitary enlargement or tumor development in rats (14, 18, 19). These changes are inhibited by a potent prolactin suppressor or an antiestrogen (13, 20). Thus, the pituitary gland and epididymis were evaluated as biological markers of 17β-estradiol or testosterone action, respectively, following the pretreatments. The body weight in tumor-bearing male rats did not differ from that in non-tumor-bearing controls. Although body weights in castrated and in castrated and 17β-estradiol-treated hosts were smaller than that of intact male hosts, the rats in all groups appeared to be in good condition. In the comparison of relative tumor weights, the differences between groups were sustained. For these reasons, we feel that the influence of the general condition of tumor-bearing rats on the grafted tumor growth would have been minimal. From these findings, it has been interpreted that the pretreatment with castration alone or in combination with 17β-estradiol had induced an alteration of the hormonal condition of tumor-bearing hosts, and 17β-estradiol had acted as an estrogenic agent in the present study. A close relationship between the tumor weight and the serum steroid levels was observed. There were significant decreases in the tumor growth related with decreasing testosterone levels or increasing 17β-estradiol levels, respectively.

Although the mechanism of the antitumor effect of 17β-estradiol treatment is unknown in the present study, it might exert its effect on tumor cells in a dual way, either directly and/or indirectly via pituitary prolactin secretion. In general, estrogen acts on its target tissues, such as the uterus, mammary gland, and pituitary gland, through estrogen receptors (21–23). We have recently observed a suppression by antiestrogen of estrogen effects on the stimulation of the pituitary gland and partial reversion of 17β-estradiol-induced growth inhibition of
transplantable rat pancreatic carcinoma. While the pancreas has not been widely recognized as one of the steroid target tissues, estrogen receptor or estrogen binding protein has been shown in normal pancreas of several species, human fetal pancreas and human pancreatic carcinomas (3-7). Grossman et al. (6) pointed out a biological importance of steroid binding protein in the normal function of pancreatic acinar cells of rats. On the other hand, prolactin has been shown to stimulate insulin secretion and to induce increased pancreas weights of mice (24, 25). Existence of insulin receptors in isolated mouse pancreatic acini has been reported (26). An interaction of prolactin with estrogen under special conditions might inhibit tumor growth by promoting functional differentiation of acinar cells. In the present study, estrogen and progesterone receptors in two cases of DSL-2 tumor in intact male hosts (Experiment 1) were examined, but these values were low levels (0.9 and 0.5 fmol/mg cytosol protein, or 6.0 fmol/mg cytosol protein and negative in each sample).5

Recently, Reddy and Schally (27) have shown the inhibitory effect of an analogue of luteinizing hormone releasing hormone on the growth of pancreatic carcinoma and suggested that the effect was mediated by suppressing androgen secretion. Thus, the antitumor effect of castration seems mainly due to decline in the stimulatory effect of testosterone rather than due to a direct effect of gonadotropin by itself.

Our recent study has shown the modulation by sex hormones of the development and growth of azaserine-induced preneoplastic lesions of pancreas in Fischer rats (8). It was unknown whether malignant cells that acquired autonomy in growth would still preserve such hormone responsiveness. However, the present study provides evidence that a transplantable rat pancreatic carcinoma is responsive to host hormonal milieu. Thus, androgens and estrogens may play key roles as modulators, promoters, and inhibitors, in the whole process of pancreatic carcinogenesis including the early to the advanced stages of malignant progression.

Serum activities of both amylase and lipase were higher in tumor-bearing intact male hosts than in non-tumor-bearing controls. The transplantable tumor, morphologically diagnosed as acinar cell carcinoma, retained acinar cell function. This finding is consistent with reports by Reddy et al. (16, 28) showing production of amylase and lipase by chemically induced transplantable rat pancreatic carcinomas. The enzymes were demonstrated in the serum and culture media. Moreover in the present study, among tumor-bearing hosts, both the amylase and lipase activities were clearly increased in proportion to the tumor weights. Accordingly, the relative decrease in both enzymic values in pretreated hosts has been considered to be causally related to impaired tumor growth that resulted from the response of malignant cells to the altered host condition. These data indicate that the transplantable rat pancreatic carcinoma was a functional tumor with enzyme secretion quantitatively related to the proliferation of malignant cells.

The serum enzymic activities reflect the production of both endogenous enzyme and enzyme from the grafted tumor. Altered host hormonal conditions induced by the pretreatment might also have influenced the function of the pancreas in situ, i.e., synthesis and/or secretion of enzymes (6), especially when the tumor volume was small. Lower serum enzyme levels observed in castrated hosts given 17β-estradiol were probably caused by hormonal effects on both the pancreas in situ and the grafted tumor in hosts. Serum amylase and lipase activities in castrated male rats without tumors were 616 ± 15 IU/dl and 0.43 ± 0.26 Sigma-Tietz units (29), which were lower than those in intact controls. The capability of cells producing enzyme might be changed under various hormonal conditions. This supposition may be supported by our recent observation that estrogen treatment influenced cell activity producing enzymes in transplantable rat pancreatic carcinoma and the ovariectomized female rat pancreas.4 In addition, a possibility for control mechanism in the interaction between endogenous and exogenous enzyme may arise. However, when under a higher enzymic activity by grafted tumor, the contribution by pancreas in situ seems minor.

It is particularly interesting that the malignant pancreatic acinar cells still retained physiological function, and its growth was responsive to the host hormonal environment. Studies of the growth of human pancreatic cancers in nude mice have suggested that testosterone stimulates their growth (30, 31). The rat transplantable tumor system, thus, may be useful for studying the mechanism of regulation of the growth and differentiation of malignant pancreatic cells by hormones.

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