Effects of Estrogen on Growth of Androgen-responsive Rat Prostatic Tumor (R 3327)

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Abstract. It has been known that estrogen has synergistic effects with androgen on growth of normal male accessory sex organs of rats. The present study was therefore undertaken to examine the effects of estrogen on androgen-responsive rat Dunning R 3327 prostatic tumor. The weight of male accessory sex organs was suppressed by estrogen on growth of treatment, but synergistic effects of estrogen and androgen on these organs were seen following combined treatment with androgen and estrogen. In contrast to the effects of estrogen on accessory sex organs, estrogen influenced a R 3327 tumor only in the negative direction regardless of whether androgen was injected simultaneously or not.

When the dihydrotestosterone injection was reduced from 500 to 100 µg/rat/day after the tumor appeared as subcutaneous nodules, the weight of the accessory sex organs was similar to that of the control animals. However, this amount of dihydrotestosterone increased tumor growth equally when compared to those treated with a pharmacological dose of dihydrotestosterone. Therefore, the response of R 3327 tumor to androgen was different from that of the accessory sex organs.

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ALTHOUGH the administration of estrogen to adult male rats evokes regressive changes in target tissues of androgen such as the reproductive organs, estrogen has synergistic effects on prostate and seminal vesicle of castrated testosterone-treated animals. [1–5]. The synergistic effects of estrogen on androgenic action may be explained, in part, by increased secretion of prolactin [6], but mostly it seems to be attributable to the direct action of estrogen on the target tissues of androgen [7]. It has been reported that estrogen causes increased permeability of cell membrane [8], interaction with androgen receptor [9], and an increase in DNA synthesis [10]. Together with these various effects, treatment with estrogen increases the weight of prostate and seminal vesicle in castrated testosterone-injected animals.

It has been reported that pathological growth of androgen-responsive tissues is also influenced by estrogen [11]. Benign hyperplasia of canine prostate evoked by androgen is accelerated by the addition of estrogen [12, 13]. It was reported that the growth of androgen-responsive mouse mammary tumor (Shionogi Carcinoma 115) was stimulated by estrogen [14]. To clarify the effects of estrogen on cancerous prostate, the present study was undertaken to examine the effects of estrogen on the growth of transplantable androgen-responsive rat Dunning R 3327 prostatic tumor (R 3327 tumor, [15]).

Materials and Methods

Animals

Copenhagen rats were donated by the National Cancer Institute (Bethesda, MD, USA) and have been maintained by inbreeding. Male Copenhagen rats 8 to 10 weeks old, weighing approximately 180 g, were used as recipients of the tumor.

Tumor

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R 3327 tumor was donated by Papanicolaou Cancer Research Institute (Miami, FL, USA) and was then maintained by successive transplantations. Sensitivity of the tumor to androgen was checked by the difference in growth in male and female rats in each generation. A small piece of tumor, of approximately 5 mg wet weight, was implanted subcutaneously with a trocar in the right flank region of animals anesthetized with ether. After the tumor became palpable, three dimensions of the mass were measured with calipers and the tumor volume was calculated according to the formula \( L \times W \times H \times 0.5236 \) [16]. Castration was performed via the scrotal route under ether anesthesia one week before tumor implantation.

**Hormone Treatment**

5α-Dihydrotestosterone propionate and estradiol-17β benzoate were dissolved in 30% ethanol-sesame oil. Daily subcutaneous injections of hormones (0.1 ml per animal) were administered from the day of tumor transplantation to 18 h before sacrifice. Non-castrated rats which received vehicle were used as the control.

**Measurement of serum level of hormones**

Serum testosterone, dihydrotestosterone, and estradiol-17β were determined by radioimmunoassay, using assay kits for testosterone and estradiol-17β (DPC, Los Angeles, CA, USA) and rabbit anti-5α-dihydrotestosterone-11-hemisuccinate-bovine serum albumin antibody for dihydrotestosterone.

**Statistical analysis**

Statistical analysis was performed by Student's t-test.

**Results**

**Effects of dihydrotestosterone and estradiol-17β separately or in combination on tumor growth and weight of accessory sex organs**

Approximately three months after tumor transplantation, the tumor transplanted into control male animals appeared as a subcutaneous nodule 100 percent successfully transplanted, and then linear growth was observed (Fig. 1). When transplanted into female rats, a small nodule appeared in almost all animals after a similar length of time, after which the size remain unchanged, and the differences in growth in male and female animals indicated the androgen-responsive growth of tumors. The injection of estradiol-17β did not induce any growth of the tumor after the initial appearance as subcutaneous nodules during the experimental period. Treatment with dihydrotestosterone (500 μg/rat/day) did not change the time of the appearance of the tumor, but significantly enhanced its growth thereafter. The addition of estradiol-17β to dihydrotestosterone did not change the time of the tumor appearance either, but thereafter suppressed growth when compared with that treated with dihydrotestosterone alone, and the rate of suppression induced by...
Fig. 2. Effects of hormone treatments on weights of R 3327 tumor, ventral and dorsolateral prostates, and seminal vesicle. Tumor-bearing animals shown in Fig. 1 were sacrificed five months after tumor transplantation and weights of the respective organs were measured (upper). Weight of seminal vesicle are the sum of bilateral organs without fluids. The numbers on the abscissa indicate the experimental groups described in the legend to Fig. 1. Data are shown as M±S.E. of percent of control (Group 1). Actual weights of the organs in the control are as follows: tumor: 4.7±0.57 g; ventral prostate: 303±12 mg; dorsolateral prostate: 283±21 mg; seminal vesicle: 358±16 mg. The statistical analysis between groups is shown (middle, lower). *P<0.05, **P<0.01.
Fig. 3. Effects of hormone treatments on morphology of R 3327 tumor. Five months after tumor transplantation, the tumor was excised. (A) control, (B) dihydrotestosterone 500 μg injection, (C) dihydrotestosterone 500 and estradiol-17β 50 μg injection. H & E staining. x200.
Table 1. Hormones in sera from control and hormone-treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Testosterone (ng/ml)</th>
<th>Dihydrotestosterone (ng/ml)</th>
<th>Estradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (12)</td>
<td>1.29±0.16</td>
<td>0.19±0.02</td>
<td>15.2±1.7</td>
</tr>
<tr>
<td>E₂ 50μg (5)</td>
<td>0.22±0.04</td>
<td>0.21±0.08</td>
<td>2403±256</td>
</tr>
<tr>
<td>DHT C 500μg (11)</td>
<td>N.D.</td>
<td>3.50±0.42</td>
<td>15.3±2.0</td>
</tr>
<tr>
<td>DHT 500–100μg (11)</td>
<td>N.D.</td>
<td>3.12±0.42</td>
<td>10.5±2.3</td>
</tr>
<tr>
<td>DHT 500+E₂ 5μg (9)</td>
<td>N.D.</td>
<td>4.88±0.73</td>
<td>108±19</td>
</tr>
<tr>
<td>DHT 500+E₂ 50μg (8)</td>
<td>N.D.</td>
<td>2.68±0.45</td>
<td>1038±155</td>
</tr>
<tr>
<td>DHT 500+E₂ 500μg (8)</td>
<td>N.D.</td>
<td>2.53±0.54</td>
<td>11600±850</td>
</tr>
</tbody>
</table>

a) Numbers in parentheses are the number of rats used for determination. b) estradiol-17β  c) 5α-dihydrotestosterone d) M ± S.E. e) not determined.

Effects of hormone treatments on histological appearance of tumors

R 3327 tumor grown in control animals showed a well-differentiated adenocarcinoma, and glands contained colloidal substances. Beneath the glands some spindle-shaped myoepithelial-like cells were dispersed. The glands were separated by fibrous connective tissues. There were a few bizarre polygonal cells in the stroma (Fig. 3). Following dihydrotestosterone treatment, the tumor showed a similar pattern to that in the control rats except that increased glandular growth and more colloidal substances were noticed.

500–100 μg dihydrotestosterone injection did not remarkably change the histological structure of the tumor when compared with that following 500 μg dihydrotestosterone treatment (data not shown). With the addition of estradiol-17β to dihydrotestosterone, the tumor was seen to be composed of small acini with less secretion than the control, and consequently the area of fibromuscular connective tissues increased. These changes were apparent when the amount of estradiol-17β was increased.

Serum hormone levels

Serum testosterone, dihydrotestosterone and estradiol-17β were measured at sacrifice (Table 1). Estradiol-17β injection did not suppress the level of dihydrotestosterone. Dihydrotestosterone injection increased serum dihydrotestosterone, but had no effect on the level of estradiol-17β. Combined injection with dihydrotestosterone and estradiol-17β increased serum dihydrotestosterone. Although the measurement of serum hormone levels was performed only at one point, the data confirmed endocrine environment evoked by hormonal manipulation.

Discussion

Synergistic effects of estrogen with androgen on male accessory sex organs have been observed not
only with regard to function but also proliferation and growth. An increase in the amount of fructose in seminal vesicle of mice and of citric acid in lateral lobe of rat prostate after simultaneous injection of androgen and estrogen are examples of functional changes [2, 3]. It was reported that estrogen enhanced the proliferating effects of androgen observed as an increase in the weight of ventral and dorsolateral prostate and seminal vesicle in castrated rats [2, 4, 5]. An increase in prostate weight was noticed following the administration of dihydrotestosterone with estradiol-17β to young castrated dog [12]. Estrogen itself caused proliferative stimulation of glandular epithelia [10]. Estrogen was reported to increase the androgen receptor in nucleus of canine prostate, potentiating androgen action [9].

In the present study, the addition of estradiol-17β to dihydrotestosterone significantly increased the weight of the ventral and dorsolateral prostate as well as seminal vesicle. The amount of increase in prostate and seminal vesicle after simultaneous injection of androgen and estrogen differed from that reported by others [2, 5]. In the latter, the increase in the weight of seminal vesicle was more evident, and this may be explained by differences in the species of animals and hormone treatment [17].

In contrast to synergistic effects of estrogen on male accessory sex organs, the growth of R 3327 tumor was reduced by adding estrogen to androgen. Since estradiol-17β alone suppressed tumor growth completely, it should be stressed that estrogen influenced R 3327 tumor only in a negative direction even when androgen was injected simultaneously. Receptors for androgen and estrogen have been demonstrated in R 3327 tumors [18, 19]. There was no progesterone receptor in R 3327 tumor [20]. The role of estrogen receptor in R 3327 tumor seems to be different from that in female estrogen target organs, since one of the actions of estrogen receptor is synthesis of progesterone receptor, as shown in mammary tumor cells [21].

Daily injection of 100 µg of dihydrotestosterone maintained the weight of male accessory sex organs at the level of those in control animals. However, the growth of R 3327 tumor in dihydrotestosterone 100 µg-injected rats was significantly higher than that in control rats and similar to that following a pharmacological dose of dihydrotestosterone 500 µg/rat/day. The R 3327 tumor appeared as a palpable subcutaneous nodule at the same time in dihydrotestosterone 500 µg-injected and control rats. Therefore, the androgen requirement for maximum growth of the tumor seemed to be different from that of male accessory organs. Previously it was reported that the effect of continuous treatment with LHRH agonist on growth of R 3327 tumor was greater than pulse type administration of a large amount of agonist, opposite to the effect on male accessory sex organs of rats [22]. These results suggest that the response to androgen is different in androgen-responsive tumor from that in normal target organs, and this is explained, at least in part, by the different in the mode of delivery with androgen.

Histological findings in various groups showed a correlation with tumor sizes which respond to hormonal manipulation. No obvious changes in the structure of tumor components was noticed, suggesting that estrogen did not significantly change the components in the structure of the R 3327 tumor.

Acknowledgments

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References

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EFFECTS OF ESTROGEN ON R 3327


