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Direct Effects of Oestradiol on Growth and Morphology of the Dunning R3327H Prostatic Carcinoma

L. Dæhlin[1,4], A. Bergh[2] and J.-E. Damber[1,3]

[1]Department of Urology and Andrology, [2]Department of Pathology and [3]Department of Physiology, University of Umeå, Umeå, Sweden, and [4]Division of Urology, Department of Surgery, University of Bergen, Bergen, Norway

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Summary. Male Copenhagen x Fischer F_1 rats were implanted with Dunning R3327H prostatic carcinoma, castrated when the tumours became palpable and were then treated with testosterone, testosterone in combination with oestradiol or oestradiol alone for four weeks. Treatment with oestradiol produced the smallest tumours. The testosterone-stimulated growth of tumours was inhibited by oestradiol. The adenocarcinoma was moderately to well-differentiated. Morphometric analysis of the composition of the tumours showed that oestradiol stimulated tumour stroma and inhibited glandular epithelium. These effects were produced concomitantly with decreased overall tumour growth. Testosterone stimulated all cell types of the tumour.

Key words: Morphometry, Oestradiol, R3327H prostatic carcinoma, Testosterone.

Introduction

The main therapeutic effect of oestrogen in patients with prostatic carcinoma is considered to be due to arrest of testicular testosterone production. However, inhibitory effects of oestrogen on the prostatic tumour have recently been reported [4, 10, 15]. The transplantable Dunning R3327H rat adenocarcinoma is a widely used model for the study of prostatic carcinoma [8]. It is histologically and histochemically similar to human prostatic adenocarcinoma [13] and is androgen-dependent for its growth [16]. In a previous study we reported that oestradiol has a direct inhibitory effect on the testosterone-stimulated growth of the Dunning R3327H carcinoma and stimulated tumour blood flow [4].

The present investigation was undertaken to study whether oestradiol has any additive inhibitory effect on tumour growth when compared to castration alone. The morphological studies were done to elucidate the effects of oestrogen on the different tissue components of the Dunning R3327H carcinoma.

Materials and Methods

Animals, Treatments and Tumour Volumes

A 1 mm^3 core of R3327H adenocarcinoma was implanted bilaterally into each flank of 30 first-generation male offspring of Copenhagen male x Fischer female rats. The procedure was carried out at the Papanicolaou Cancer Research Institute, Miami. The animals were then flown to the University of Umeå, caged 2–3 and kept in a controlled environment. Rat pellets and water were freely available. Three to four months after implantation of tumours all rats, when weighing 380–450 g, were castrated under ether anaesthesia. One half of the animals were studied without further treatment (C), while the remaining rats were randomly allocated to one of three treatment groups. Starting on the day of castration, each group of 5 animals received one of the following treatments by daily subcutaneous injections for four weeks:

Group I; testosterone proprionate 100 μg = C + T

Group II; testosterone proprionate 100 μg with oestradiol benzoate 50 μg = C + T + E_2

Group III; oestradiol benzoate 50 μg = C + E_2

Tumour volumes were calculated as previoulsy described and the selected dose of testosterone has been shown to produce tumour growth almost identical to that in intact animals [4]. In the batch of rats concerned, tumour growth in intact animals corresponded to that of testosterone-supplemented animals.

Hormones and chemicals were purchased as previously reported [4].

Morphologic Examinations of Tumours

The left and right tumours were weighed, pieces of approximately 1 cm^3 being randomly chosen both from the peripheral and central parts of the tumour, fixed in Bouin solution and embedded in paraffin. Tumour morphology was investigated in 5 μm thick sections stained with hematoxylin-eosin and van Gieson elastin.

The volume density of tumour epithelium, stroma and acinar lumina was determined at 250x magnification using a square lattice mounted in the eyepiece of a light microscope and by counting the number of testpoints over each of these tissue compartments [17].

When expressing morphological data in absolute values, prostatic density was set to 1.0 g/ml. The cross-sectional area of randomly chosen epithelial and stroma cell nuclei was measured using a

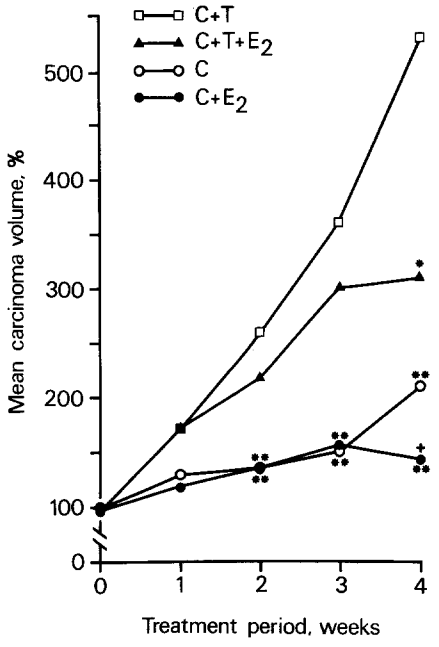


Fig. 1. Growth plots of the R3327H prostatic carcinoma after different hormonal treatments. For abbreviations, see text. ** = $p < 0.01$, * = $p < 0.05$, when compared to testosterone-supplemeneed rats ($C + T$). + = $p < 0.01$, when compared to castration alone (C)

drawing tube, encircling the cell nuclei over a digitizer table connected to a computer [1]. One hundred nuclei of each type and tumour were measured. These measurements were performed at 1,000x magnification.

Statistics

Values are expressed as mean ± SEM unless otherwise indicated. Comparisons between groups were made by the Mann-Whitney U test [12]. A p-value of less than 0.05 was considered as statistically significant.

Results

Tumour volumes in % of value at start of treatment are given in Fig. 1. When compared to testosterone-supplementation, the growth of tumours after castration alone or with additional oestradiol injection was significantly ($p < 0.01$) decreased after 2, 3 and 4 weeks of treatment, while oestradiol in combination with testosterone inhibited tumour growth significantly ($p < 0.05$) after 4 weeks of injection. Oestradiol injection in castrated rats resulted in significantly ($p < 0.01$) smaller tumour volumes than after castration alone at 4 weeks of treatment. Carcinoma volumes at start of treatment in castrated, testosterone-supplemented, testosterone combined with oestradiol and oestradiol-injected animals were 3.68 ± 0.48, 3.03 ± 0.96, 3.90 ± 0.69 and 3.49 ± 0.85 ml, respectively. There was no significant difference between the groups.

In testosterone-supplemented rats the tumour was composed mainly of small, closely packed tumour glands with small lumina. The tumour epithelium was approximately two-layered and crowded with small cell nuclei, and mitoses were present. The stroma was not well developed and contained only few muscle cells. After oestradiol treatment alone the tumour was composed mainly of stroma with well-developed smooth muscle cells. The tumour glands contained large lumina and the epithelium was one-layered and flat. The number of epithelial nuclei and the number of mitoses appeared to be reduced but nuclear sizes were larger than in tumours of testosterone-supplemented animals. The general morphology of tumours from animals given the combination of testosterone and oestradiol was similar to that after oestradiol treatment alone. However, the tumour epithelium was more developed with numerous large nuclei; the acinar lumina were smaller in animals given the combined treatment (Figs. 2–4).

The observations were subjected to morphometric analysis (Table 1). The addition of oestradiol to testosterone induced a significant ($p < 0.05$) increase of stroma volume density, whereas the volume density of glandular epithelium decreased significantly ($p < 0.05$). The volume density of glandular lumina was significantly ($p < 0.05$) increased and the nuclear sizes of tumour stroma and glandular epithelial cells increased significantly ($p < 0.05$) by addition of oestradiol to testosterone supplementation. Testosterone stimulated the glandular epithelium as demonstrated by the significant ($p < 0.05$) increase of volume density of glandular epithelium after addition of testosterone to oestradiol treatment. The total volumes of the different parts of tumours after treatments with testosterone, testosterone in combination with oestradiol or oestradiol alone are shown in Table 2. In rats given the combined treatment of testosterone and oestradiol, the total volume of tumour stroma was significantly ($p < 0.05$) larger than in animals injected with testosterone alone. Concomitantly, the total volume of glandular epithelium tended to decrease on addition of oestradiol to testosterone-supplementation.

The total volumes of tumour stroma and glandular epithelium were significantly ($p < 0.05$ and $p < 0.01$, respectively) larger after the combined treatment with testosterone and oestradiol when compared to oestradiol injection alone.

Discussion

Oestrogen inhibits the overall growth of Dunning prostatic carcinoma by a direct effect, since tumour growth in castrates was inhibited with or without testosterone supplementation. This agrees with previous reports showing that oestradiol inhibits the testosterone-stimulated growth of Dunning R3327H tumours [4] and the transplantable human prostatic carcinoma PC82 in nude mice [14], and also with results achieved after treating tumour-bearing castrated rats with diethylstilboestrol [10]. The dose of diethylstilboestrol which Shessel et al. [10] used was about seven times higher than in the present work. Since the oestrogenic potency

Table 1. Volume densities of tumour stroma, glandular epithelium and glandular lumina and the nuclear sizes of stroma and epithelium after different hormonal treatments of Dunning R3327H – implanted rats

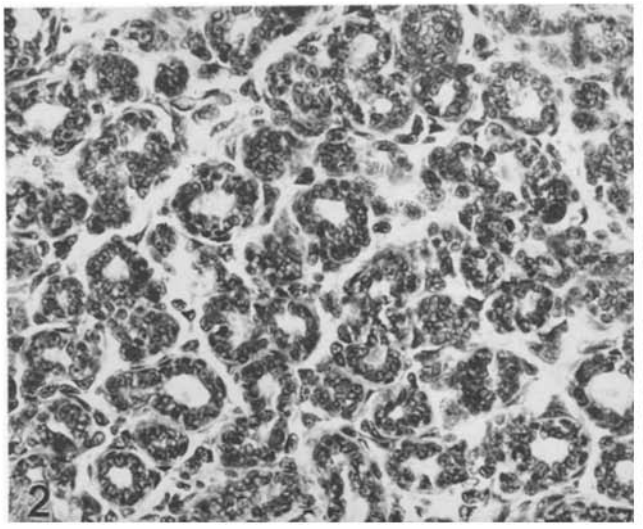
Group	*n*	Volume density (%)			Nuclear size (μm^2)	
		Stroma	Epithelium	Lumen	Stroma	Epithelium
C + T + E_2	10	47.1 ± 7.8[a]	34.2 ± 3.9[a,b]	18.9 ± 5.0[a]	38.5 ± 5.6[a]	26.4 ± 5.7[a]
C + T	10	32.9 ± 2.7	57.4 ± 3.2	9.7 ± 2.6	23.6 ± 5.8	13.4 ± 2.9
C + E_2	10	49.8 ± 5.7	25.4 ± 3.1	24.6 ± 3.2	33.6 ± 1.9	20.9 ± 1.7

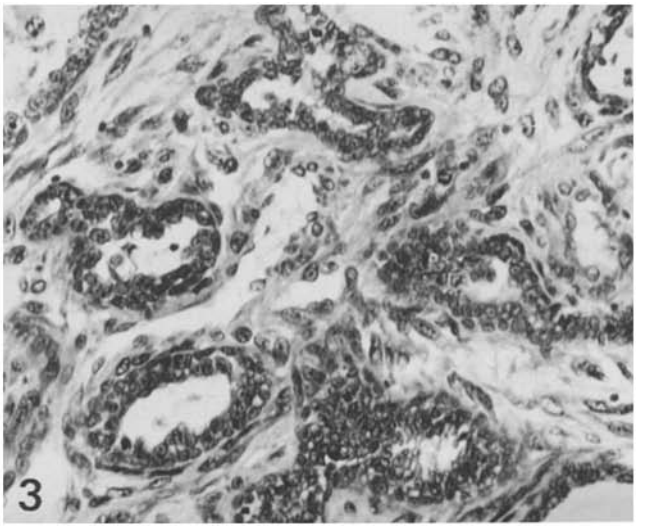
For abbreviations, see text. Mean values ± SD are given. [a] = $p < 0.05$ when compared to testosterone-substituted (C + T) rats; [b] = $p < 0.05$ when compared to oestradiol treatment alone (C + E_2). *n* = number of tumours studied

Table 2. Total volume (ml) of stroma and glandular epithelium in R3327H prostatic tumours after different hormonal treatments

Group	Stroma	Glandular epithelium
C + T + E_2	10.5 ± 2.3 [a,b]	7.2 ± 1.2[d]
C + T	6.1 ± 1.6	11.0 ± 3.1
C + E_2	4.4 ± 1.1	2.4 ± 0.5

For abbrevations, see text. Mean values ± SEM are given. [a] = $p < 0.05$, when compared to testosterone-supplemented animals (C + T); [b] = $p < 0.05$, [d] = $p < 0.01$, when compared to oestradiol treatment alone (C + E_2)





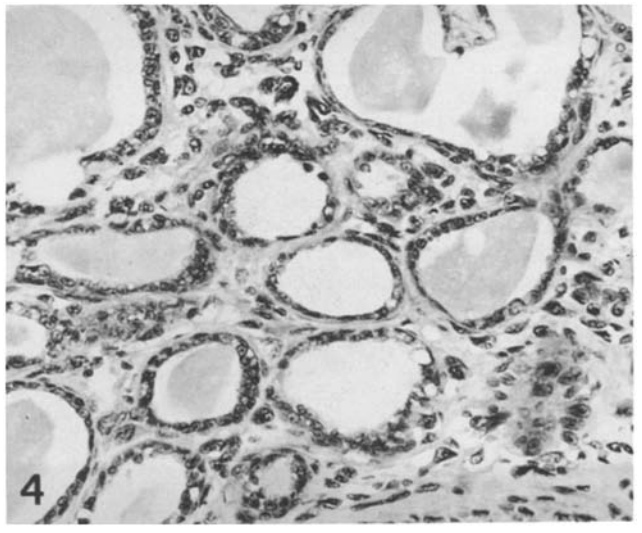


Fig. 2–4. Representative sections from R3327H prostatic carcinoma in castrated rats treated with testosterone (**Fig. 2**), testosterone in combination with oestradiol (**Fig. 3**) or oestradiol injection alone (**Fig. 4**), x250. For details, see results

of diethystilboestrol and oestradiol are comparable [5] the present data suggest that the overall growth of Dunning R3327H carcinoma is more susceptible to oestrogen than previously realized.

This is the first report using morphometric techniques for evaluation of oestrogenic effects on the R3327 tumour. Oestradiol increased the volume density and the total volume of tumour stroma as well as stromal nuclear size. The volume density and total volume of glandular epithelium were, however, decreased. These findings suggest a direct action of oestradiol on prostatic carcinoma by stimulation of the stroma and by an inhibitory effect on the epithelium, leading to inhibition of the overall growth of the carcinoma.

Since the stroma is the vessel-containing part of the tumour, the stimulatory effect of oestradiol on the volume density of tumour stroma offers an explanation for our previous findings of stimulated blood flow to R3327H carcinoma after oestrogen treatment [3]. The mechanims of action behind the stimulation of stroma by oestrogen administration is not understood. Krieg et al. [6] reported that the oestrogen receptor in human benign hyperplasia and normal prostate was preferably assayed in the stroma. Stromal protein synthesis in the human prostate is inhibited by tamoxifen administration [7]. Since the Dunning R3327H carcinoma and human benign prostatic hyperplasia contain oestrogen receptors [2, 9, 11], it is proposed that the oestrogenic effects on the tumour stroma are promoted by a receptor-mediated mechanism.

The inhibited growth of the Dunning R3327H carcinoma by oestrogen administration may be of relevance to the management of prostatic carcinoma patients, providing support for the notion that addition of oestrogen to castration might result in an enhanced inhibitory effect on human prostatic carcinoma. This is consistent with a recent work [18] reporting that the proportion of prostatic carcinoma patients with progression on first-line treatment was lower with diethylstilboestrol than after medical castration through the administration of an LHRH-agonist.

In summary, the present study further demonstrates the importance of direct effects of oestradiol on prostatic carcinoma. Oestradiol stimulates the stroma and inhibits the glandular epithelium of prostatic carcinoma, resulting in an inhibited overall growth of the tumour.

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L. Dæhlin, M.D.
Division of Urology
Department of Surgery
N-5021 Bergen
Norway