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Blood Flow in the Dunning R3327H Rat Prostatic Adenocarcinoma; Effects of Oestradiol and Testosterone

L. Dæhlin[1] and J.-E. Damber[1,2]

[1]Department of Urology and Andrology and [2]Department of Physiology, University of Umeå, Umeå, Sweden

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Summary. Copenhagen x Fischer F_1 rats were implanted with Dunning R3327H prostatic adenocarcinoma and studied over a period of three weeks. The tumour volumes in intact and testosterone supplemented castrated rats showed parallel increases. After castration alone the tumour volumes decreased. Treatment of castrates with oestradiol and testosterone combined produced an arrest of tumour growth, suggesting that oestradiol had a direct inhibitory effect on tumour growth. Blood flow in tumours was measured using the microsphere technique. In intact rats, tumour blood flow per unit of weight decreased with increasing weight of tumours and blood flow in peripheral parts was higher than in central parts of large tumours. Oestradiol in combination with testosterone increased tumour blood flow.

Key words: R3327H prostatic carcinoma, Blood supply, Testosterone, Oestradiol.

Introduction

Prostatic blood flow is essential for normal prostatic growth and function. Testosterone substitution in castrated Sprague-Dawley rats stimulates prostatic blood flow and administration of oestradiol, ethinyl oestradiol or diethylstilboestrol inhibits this effect [8]. It is not known whether the direct effect of oestrogen produces a blood flow in the prostate of normal rats which corresponds to the blood flow in neoplastic prostatic tissue.

One of the few animal tumour models for prostatic adenocarcinoma is the Dunning R3327 rat carcinoma transplanted into Copenhagen x Fischer rats [11]. This tumour is of dorsolateral prostatic origin, developed spontaneously in an aged animal and has histological and biochemical similarities to the human prostatic carcinoma. Voigt and Dunning [23] showed that the rat tumour is androgen dependent for its growth and contains 5α-reductase. Acid phosphatase, androgen- and oestrogen receptors were later demonstrated [14, 5, 16], indicating a close similarity between this rat tumour and human prostatic adenocarcinoma.

The present investigation was undertaken to study possible effects of oestradiol on the growth rate and blood flow in the Dunning R3327H prostatic adenocarcinoma.

Materials and Methods

Animals, Treatment and Tumour Volumes

A 1 mm^3 core of R3327H adenocarcinoma was implanted bilaterally into each flank of the first generation male offspring of Copenhagen and Fischer rats when the hybrids had reached an age of 6–10 weeks. The procedure was carried out at the Papanicolaou Cancer Research Institute, Miami, and the animals were then flown to the University of Umeå, and kept in a controlled environment. Rat pellets and water were freely available.

Four to five months after implantation of tumours when the weight of rats reached 400 (385–413) g, the animals were randomly allocated to one of four groups. The rats in three groups were castrated by means of a scrotal incision under ether anaesthesia. The various groups were:

Group A: 11 rats; intact animals injected with sesame oil.
Group B: 10 rats; castrated and injected with sesame oil = V.
Group C: 14 rats; castrated and injected with testosterone proprionate 100 μg = T.
Group D: 14 rats; castrated and injected with testosterone proprionate 100 μg with oestradiol benzoate 50 μg = $T + E_2$

All animals received two daily subcutaneous injections each of 0.05 ml in different sites over a period of three weeks, starting on the day of randomization and with the last injection given 13 (12–14) hours before the blood flow measurements.

From the first day of treatment three perpendicular tumour diameters in all animals were measured weekly with micro-calipers under ether anaesthesia. Tumour volumes were estimated by using the formula of an ellipsoidal mass [15]. Volume index at different treatment times was = tumour volume/tumour volume at start of treatment x 100, %.

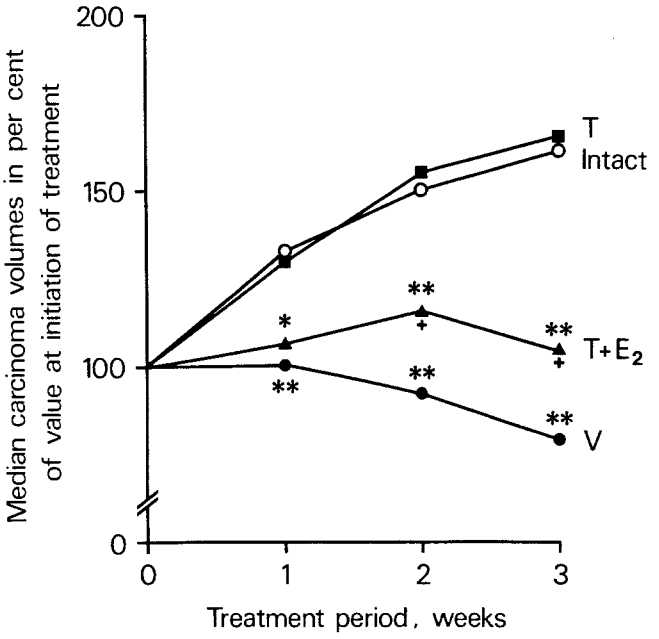

Fig. 1. Growth plots of the R3327H Dunning rat adenocarcinoma under various hormone conditions. The animals were randomly allocated to one of four groups; intact animals; castrated and vehicle injected (V); castrated and testosterone supplemented (T); castratet, testosterone supplemented and treated with oestradiol ($T + E_2$). Daily s.c. injections were given for three weeks. Tumour diameters were measured weekly with micro-calipers during the treatment period and volumes were calculated as a percentage of the value at the start of treatment. Median values are given. ** = $p < 0.01$, * = $p < 0.05$, when compared to the T-supplemented group, + $p < 0.05$, when compared to castration alone

Measurement of Nutritive Blood Flow Using Radioactive Microspheres

Anaesthesia was induced by intraperitoneal doses of 32 μg fentanyl citrate with 1.0 mg fluanisone (Hypnorm^R, AB Leo, Sweden), 1.0 mg diazepam (Stesolid^R, Dumex, Denmark) together with 20 μg atropine. During experiments the animals were kept supine on a heated pad. Blood flow to various organs was measured using radioactive microspheres labelled with cerium-141 (15.5 ± 0.3 μm diameter) and purchased from NEN, USA. The microsphere technique for measuring blood flow was carried out as described earlier [9]. After subsequent collection of a blood sample from the carotid artery, the tumours were carefully dissected out, weighed and divided into approximately four equal parts. Small pieces from the middle parts of cut-surface edges were processed as samples from the central parts and small pieces were randomly cut from tumour surfaces and processed as samples from the peripheral parts of tumour. The topical separation of samples was only achieved with difficulty in the smallest tumours. In addition, the following organs were dissected out; ventral prostate, seminal vesicles, kidneys and representative parts of the iliopsoas muscle.

Blood flow values are given as: ml x (100 g tissue)$^{-1}$ x min^{-1}.

Vascular resistance: mean arterial blood pressure (mmHg)/blood flow in the tissue (ml x (100 g tissue)$^{-1}$ x min^{-1}).

The precision of the blood flow measurements at 95% confidence level were calculated according to Buckberg et al. [2].

Blood samples were centrifuged and stored at −20 °C until analysed.

Hormones

Testosterone proprionate was purchased from Schering AG (Germany) and oestradiol benzoate from Biosynth (Netherlands). Testosterone and oestradiol were dissolved in sesame oil. All other chemicals were of analytical grade and purchased from Sigma (USA).

Testosterone Assay

Testosterone concentration in plasma was analysed using radioimmunossay [6] and the within-assay coefficient of variation was 11 per cent.

Statistics

For presentation of blood flow measurements in intact rats, tumours were divided into three groups. Small = tumour volume, Tv < 3,000 mm^3 (n = 7); medium = 3,000 ⩽ Tv ⩽ 9,200 mm^3 (n = 6); large = Tv > 9,200 mm^3 (n = 8).

Median values are given with 25- and 75-percentiles. Comparisons amongst groups were made using the Kruskall-Wallis one-way analysis of variance. For comparisons between and within groups the Wilcoxon's two-sample and matched-pairs signed-ranks tests were employed. Correlation was expressed by the Spearman rank correlation coefficient, r_s [20] and regression was computed by the least squares method [21]. A p-value of less than 0.05 was considered as statistically significant.

Results

During the injection period of three weeks, the volumes of carcinomas in intact as well as testosterone supplemented castrated animals all increased ($p < 0.01$). After castration alone 16/20 tumour volumes decreased ($p < 0.05$).

Tumour volume indices are presented in Fig. 1. The growth of carcinomas in testosterone-supplemented rats was almost identical to that in intact animals. When compared to testosterone supplementation, castration alone induced significantly ($p < 0.01$) decreased growth of tumours after one, two and three weeks of treatment. Tumour volume indices in animals injected with the combination of testosterone and oestradiol were significantly smaller than in testosterone supplemented rats ($p < 0.05$ after one week; $p < 0.01$ after two or three weeks) and significantly ($p < 0.05$) larger than with castration alone after two and three weeks of treatment. For intact and castrated rats the correlation coefficients between tumour volume at initiation of treatment and volume increase during the treatment period were 0.68 and -0.60, respectively.

The weights of all tumours were plotted against their calculated volumes from diameter measurements and the correlation coefficient was 0.97 (Fig 2.).

In intact animals nutritive blood flow in both the peripheral and central parts of carcinomas was siginificantly ($p < 0.05$) higher in small tumours than in the two other weight groups, as shown in Fig. 3. Moreover, there was a significant ($p < 0.05$) difference between peripheral and central blood flows in the large tumours.

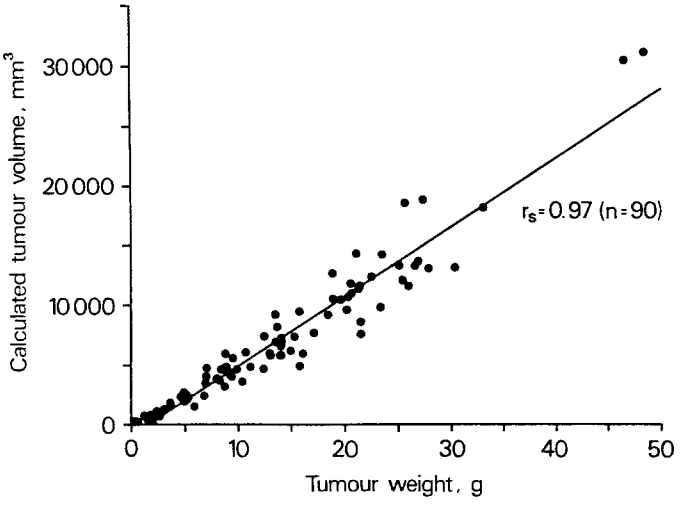

Fig. 2. Correlation between calculated volume from diameter measurements and weight of Dunning R3327H rat prostatic adenocarcinoma

Table 1. Blood flow in Dunning R3327H prostatic adenocarcinoma and corresponding tumour volume

Group	n	Blood flow ($ml \times 100g^{-1} \times min^{-1}$)		Tumour volume (mm^3)
		peripheral	central	
Intact	21	20 (7–41)	15 (6–40)	5,990 (1,272–10,240)
T	28	13 (8–35)	12 (5–18)	7,330 (4,840–12,200)
V	20	20 (14–38)	16 (10–32)	4,480 (1,490–6,620)
$T + E_2$	27	$54^{a,b}$ (15–94)	$43^{a,b}$ (12–92)	3,960 (2,270–6,810)

For abbreviations, see text. Median values are given with 25- and 75-percentiles within parentheses, n = number of tumours; a = $p < 0.01$ when compared to corresponding part of tumour in testosterone supplemented group, b = $p < 0.05$ when compared to castration alone

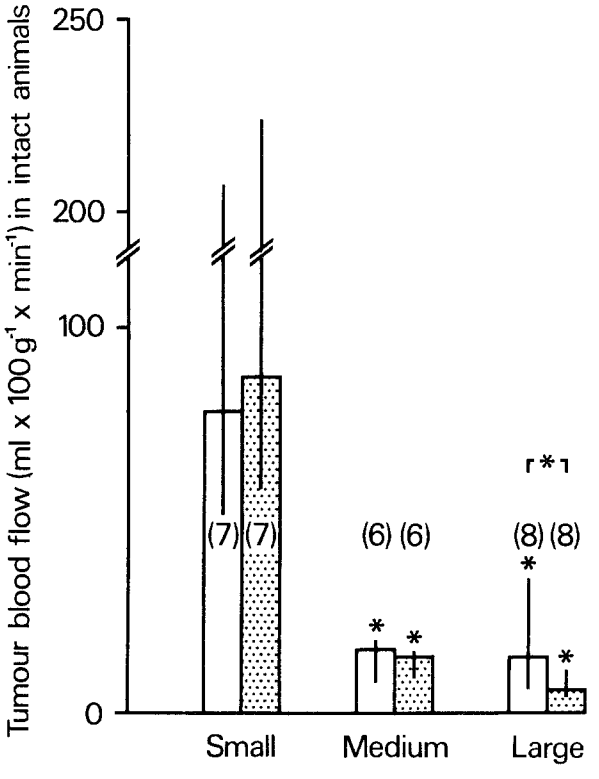

Fig. 3. Blood flow to Dunning R3327H prostatic adenocarcinoma in intact Copenhagen x Fischer F_1 rats measured using the microsphere technique. Results are given for three different volume ranges of the tumours; small = tumour volume (Tv) < 3,000, medium = 3,000 ⩽ Tv ⩽ 92,000, large = Tv > 9,200, mm^3. Open bars represents blood flow to peripheral parts, hatched bars shows blood flow of central parts of tumours Median values are given with 25- and 75-percentiles. The number of tumours within each group is given within parentheses. * = $p < 0.05$, when compared to corresponding part in the smallest tumours; ⌐*¬ = $p < 0.05$ when a comparison was made between peripheral and central parts of tumours within the same weight range

Blood flows in carcinomas of the whole material together with their volumes at the time of blood flow measurements are shown in Table 1. In castrated rats treated with testosterone and oestradiol, tumour blood flow was significantly higher than after castration alone ($p < 0.05$) or in combination with testosterone supplementation ($p < 0.01$). These statistical conclusions were confirmed when tumours of the same sizes in the three groups were selected for analysis (all tumours with volumes less than 5,700 mm^3 were selected; animals that were testosterone-supplemented, vehicle injected and treated with testosterone plus oestradiol had median tumour volumes of 2,386, 2,212 and 2,382 mm^3, respectively). When corrections were made for the small differences in blood pressure between groups by computing peripheral and central tumour vascular resistances, the statistical conclusions from blood blow measurements were confirmed.

In intact animals there was a close correlation between peripheral/central tumour blood flow and volume indices, the correlation coefficients were 0.52 and 0.77, respectively.

Mean arterial blood pressure, plasma testosterone concentration together with blood flows to the ventral prostate, seminal vesicles, kidneys as well as to iliopsoas muscle are shown in Table 2. Castration alone induced a significant reduction ($p < 0.01$) of blood flow to the seminal vesicles when compared to testosterone supplementation. Plasma testosterone concentration decreased ($p < 0.01$) after castration alone and increased ($p < 0.05$) significantly after treatment with the combination of testosterone and oestradiol as compared to testosterone supplementation.

Ventral prostatic weight (mg) was 60 (45–68) after castration alone and 311 (276–364) in the rats treated with testosterone plus oestradiol which is significantly ($p < 0.01$) lower than after testosterone supplementation, 486 (427–

Table 2. Mean arterial blood pressure (MAP), plasma testosterone concentration (test) and blood flow to different tissues in Dunning R3327H implanted rats

Group	*n*	MAP (mmHg)	test (ng/100 ml)	Blood flow to tissues (ml x 100 g^{-1} x min^{-1})			
				ventral prostate	seminal vesicles	kidneys	iliopsoas muscle
Intact	11	70 (64–75)	126 (94–168)	21 (14–33)	36 (28–72)	593 (463–800)	46 (34–55)
T	14	66 (54–74)	116 (100–129)	18 (15–21)	40 (35–51)	534 (503–590)	42 (34–46)
V	10	65 (53–73)	59.5[a] (55–61)	14 (11–17)	18[a] (15–24)	440 (369–502)	34 (31–42)
T + E_2	14	71 (65–76)	133[b] (123–152)	30 (19–38)	35 (25–42)	458 (404–668)	43 (33–53)

For abbreviations, see text. Median values are given with 25- and 75-percentiles within parentheses, *n* = number of observations
[a] $p < 0.01$
[b] $p < 0.05$ when compared to testosterone-supplemented rats

533). Castration alone induced a significant ($p < 0.01$) decrease in the weight of the seminal vesicles (mg) when compared to testosterone supplemented rats, 147 (141–150) vs. 513 (481–543). The number of microspheres per unit of weight in the right and left kidneys was closely correlated ($r_s = 0.79$), indicating the distribution of microspheres at the level of renal arteries. The median precisions of blood flow measurements for the whole material were; 8/9 per cent for peripheral/central parts of carcinomas; 13, 9 and 6 per cent, respectively for ventral prostate, seminal vesicles and iliopsoas muscle [2].

Discussion

This is the first report to deal with blood flow in the Dunning R3327 rat prostatic adenocarcinoma. In intact controls the blood flow per unit of weight was highest in the small tumours. The reason for this is unknown but similar results were obtained in other tumour models [1, 12]. We observed decreased blood flow to central parts of large tumours. This may be due to different degrees of vascularization in the peripheral and central parts of the Dunning tumour. The interstitial pressure in large mammary carcinomas of rats is elevated in central parts of the tumour, possibly due to increased capillary permeability and/or insufficient lymphatic drainage [24], and similar mechanisms may explain the decreased blood flow in central parts of the largest carcinomas in the present study.

The lack of effect of testosterone and stimulatory effect of oestradiol on R3327H tumour blood flow is not in agreement with previous observations, demonstrating that testosterone stimulates and oestradiol inhibits blood flow in the prostate in castrated, testosterone supplemented and oestradiol treated Sprague-Dawley rats [8]. There may be several reasons for this discrepancy. Firstly, tumour vessels lack a smooth muscle coat [18], which plays an important role in the blood flow regulation of normal organs. Secondly, a specific angiogenetic factor known to be present in some tumour tissues [10] is possibly lacking in normal organs since revascularization of normal tissued grafts is the result of fusion of pre-existing vessels with the host circulation. It may be so that testosterone per se interferes with tumour angiogenesis to a limited extent in contrast to oestradiol which possibly modulates the activities of tumour angiogenetic or inhibiting factors. Thirdly, the metabolic requirements of neoplastic and normal tissues may be different. Furthermore, the main action of oestrogen administration may be on tumour stroma cells thereby producing an increased blood flow per unit of weight to the tissue. The combined effects of arrested growth and increased tumour blood flow have also been observed with other treatment modalities of malignancy, e.g. by Kjartansson et al. [13] in short-term experiments with irradiated rat sarcoma.

The selected dose for testosterone supplementation maintained tumour growth alomost identical to that in intact rats. After castration alone the correlation between tumour volume at the start of treatment and volume change during the observation period shows that tumour mass was most efficiently reduced in the largest tumours. This observation may be of relevance to the controversy concerning early versus delayed treatment of prostatic carcinoma patients.

Oestradiol in combination with testosterone arrested the growth of R3327H adenocarcinoma, which suggests that oestrogen has a direct growth-inhibitory effect on prostatic carcinoma. This observation is in line with the report of Shessel et al. [19] showing that castration and treatment with diehtylstilboestrol produced lower weights in R3327G tumours than castration alone. The weekly does of diethylstilboestrol, however, was seven times higher than the dose of oestradiol given in the present work. The tumour growth

amongst animals treated with testosterone and oestradiol in the present work is comparable to data reported by Müntzing et al. [17], who injected testosterone and estramustine phosphate (100 mg per kg body weight three days a week) into castrated rats bearing R3327 tumours. Since one of the main metabolites of estramustine phosphate is oestradiol [7], it may be suggested that the growth-inhibiting effect of estramustine phosphate on the R3327 tumour is mediated, at least in part, by the metabolite oestradiol.

Our present observations of blood flow to the ventral prostate of Copenhagen x Fischer rats do not confirm the data we obtained earlier from Sprague-Dawley rats [8], perhaps because of differences in prostatic response to hormone manipulations between rat species [3].

Plasma testosterone concentration was significantly increased in rats given the combined treatment of testosterone and oestradiol, which may be due to oestradiol mediated inhibition of 5α-reductase activity in carcinoma tissue and consequently decreased testosterone metabolism [22]. Stimulated adrenal androgen production in response to oestrogen treatment is another possible explanation for the elevated testosterone level [4].

The present study demonstrates circulatory characteristics of the Dunning R3327H prostatic carcinoma. It is shown that oestradiol has a growth-inhibitiory effect which is accompanied by increased blood flow in the tumour.

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Dr. L. Dæhlin
Department of Urology and Andrology
University of Umeå
S-90185 Umeå
Sweden