Brief Communication

The Development of Prostatic Adenocarcinoma in Nb Rats following Prolonged Sex Hormone Administration

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SUMMARY

The low spontaneous incidence of grossly recognizable adenocarcinomas of the dorsal lobe of the prostate in Nb rats over 13 months old (0.45%) was increased to 18.38% in 130 rats by prolonged treatment with pellets of suitable sex hormones. Although testosterone propionate alone was effective, if the testosterone propionate was combined with treatment by estrone carcinomas of the prostate occurred after a shorter interval. Tumors tended to metastasize and transplanted readily, and all were autonomous, with a single exception that exhibited the growth pattern of a typical estrogen-dependent tumor.

INTRODUCTION

Adenocarcinoma of the prostate, a relatively common form of cancer of the aged human, may show a striking response to hormones. The condition may frequently be arrested temporarily by procedures such as castration which reduces male sex hormones, as originally demonstrated by the classic studies of Huggins and Scott, or through antagonism of the male sex hormones by estrogens, as subsequently found by Herrold. In mice and rats, however, the disease has proven to be particularly elusive in attempts to duplicate it experimentally, and it has never been produced by steroid treatment (9, 10). Spontaneously arising tumors, on the other hand, have been noted occasionally but in only a few rats (1, 2, 6). It has been reported recently that germ-free Wistar rats, which live to an exceptionally old age, have a higher incidence (17-19). Prostatic cancer, usually of a squamous cell type, may be produced in rats, however, by the direct application of chemical carcinogens to the gland (3-5, 7, 8). The lack of experimental evidence to implicate hormones as the cause of prostatic carcinoma has probably led to the intensified search for environmental or other carcinogenic factors of etiological importance in man. This report will describe for the 1st time hormonal conditions in rats that increased the low spontaneous incidence (0.45%) of grossly recognizable adenocarcinomas of the prostate to 20% with suitable treatment, and it suggests that a hormonal etiology of the condition in humans should be seriously reconsidered (13).

MATERIALS AND METHODS

The Nb strain of rats has been used in these and in other studies on tumors controlled by hormones, as previously described (15, 16). It is not pathogen free, and normal males seldom survive beyond 2 years. EP3 or TPP pellets of approximately 10 mg, made in a hand press, were inserted s.c. Whereas an EP lasted for the animal's lifetime, the TPP's dissolved more rapidly and were replaced at 6- to 8-week intervals. Since carcinoma of the prostate usually occurred in old rats, any that appeared ill or with abdominal masses were killed. However, a number dying of the disease were undoubtedly not recorded. Only rats treated for 6 months or more and with TPP replaced at least 4 times are included in this paper. Transplants were made from small pieces of tumor placed in a trochar and inserted into the back of the neck of normal or hormonally conditioned 8- to 12-week-old animals. At postmortem most organs were weighed, and metastases, which occurred frequently, were noted. In this paper only the incidence of tumors grossly distorting the gland but confirmed histologically has been reported. Rats were fed Purina chow.

RESULTS

Spontaneously arising adenocarcinomas of the prostate have been noted in only 5 rats of this colony, and 2 probably represented tumors of the seminal vesicle. All arose in rats over 1 year old. In the past 5 years 2 carcinoma of the prostate occurred in 1185 control male rats examined after 9 months of age. The 2 tumors occurred in 13- and 14-month-old rats from 409 rats of 13 months or older, representing an incidence of 0.48% in animals of this age. All spontaneous tumors were transplanted successfully and showed extensive metastases (Fig. 1). Table 1 records the occurrence of gross tumors of the prostate in rats subjected

1 Professor of Physiology, University of British Columbia.
2 A cancer unit of the National Cancer Institute of Canada which supported the earlier investigations and provided facilities for the research.
3 The abbreviations used are: EP, a pellet containing 90% estrone and 10% cholesterol; TPP, a pellet containing 90% testosterone propionate and 10% cholesterol.
The tumor incidence was markedly increased in the treated to a number of different treatments with hormone pellets. The tumor incidence was markedly increased in the treated animals. A single TPP, however, inserted at 2-month intervals over periods of 9 to 31 months did not cause carcinoma. When 2 or more pellets at a time were used, the treatment was highly effective. Following TPP treatment tumors were found in rats after an average of 59 to 64 weeks, but in EP-bearing animals, similarly treated, the tumors occurred in 48 weeks. The minimum dose of testosterone to produce a tumor was a total of 10 pellets, and the tumors occurred in 43 weeks. The minimum dose of testosterone to produce a tumor was a total of 10 pellets, and the shortest time was 27 weeks. Of the 25 tumors listed possibly starting at the margin of the seminal vesicle. In many cases urine flow was obstructed, resulting in dilation of the bladder and ureters, and infection of the kidney (Fig. 3). Primary tumors of the bladder and papillary hemorrhagic mammary carcinomas were also encountered in animals treated with EP plus TPP (11, 12). The histological appearance of all tumors whether spontaneous or induced was similar, with well-developed areas of glandular epithelial cells surrounded by varying degrees of more compact scirrhous tissue (Figs. 2, 4, 6, and 9). Although transplants might appear to be mainly scirrhous, metastases were usually of more differentiated epithelial components (Figs. 10 to 12). Squamous cell proliferation has not been seen. The surrounding prostatic tissue appeared normal, although hyperplastic, but follicles immediately adjacent to the tumor frequently were distended with cellular debris, phagocytic cells, and possible low-grade infection, with in situ changes suggesting multifocal areas of early cancer. Prostates from many other treated rats without gross changes showed early in situ lesions indicative of early carcinomatous change (9, 10). Brown pigmentation of the ventral lobe and microscopic lesions similar to those described by others (20) have been noted, but these have not progressed to gross tumor development. All transplanted carcinomas have been autonomous, and their growth could not be influenced by treatment with sex hormones. In the exceptional adenocarcinoma 52, however, the growth of the initial transplants was slow but normal by the 3rd transplant generation when maintained in EP plus TPP hosts. After this time, however, transplants would grow only in hosts after treatment that included estrogen, and such estrogen dependency has persisted for 12 generations. The tumor showed well-developed epithelial components, and the histological appearance had not changed. Some sublines have

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### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence of gross carcinoma of prostate (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of rats</th>
<th>Mean duration of treatment (wk)</th>
<th>Metastases present</th>
<th>No. of transplant lines established</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPP (1 pellet)</td>
<td>13</td>
<td>64 (48-91)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>59 (48-78)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>TPP (2 pellets)</td>
<td>30</td>
<td>57 (41-90)</td>
<td>16.8</td>
<td>64 (37-89)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>TPP (3 pellets)</td>
<td>55</td>
<td>64 (29-90)</td>
<td>20.0</td>
<td>43 (40-60)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>EP + TPP (2 pellets)</td>
<td>2</td>
<td>79</td>
<td>0</td>
<td>34 (13-52)</td>
<td>8</td>
</tr>
<tr>
<td>EP + TPP (3 pellets)</td>
<td>45</td>
<td>46 (27-62)</td>
<td>17.7</td>
<td>63 (32-76)</td>
<td>5.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Testosterone propionate pellets were replaced every 6 to 8 weeks.
<sup>b</sup> In 409 controls, ages 56 weeks or more, there were 2 spontaneous tumors (0.48% incidence); in 130 steroid-treated rats of 3 groups in the table, there were 24 induced tumors (18.38% incidence).
<sup>c</sup> Numbers in parentheses, range.
shown progression and now grow slowly in untreated male rats. Removal of the EP in males with growing tumors has led to a regression in the size of the tumors, but after 16 to 40 weeks spontaneous tumor regrowth occurred. When such regrowth was transplanted, it had changed from an estrogen-dependent to an autonomous growth, similar to the pattern of changes observed in estrogen-dependent tumors of other organs (14).

DISCUSSION

It has been possible to demonstrate that the low spontaneous incidence of prostatic carcinoma in Nb rats may be greatly increased by prolonged treatment with testosterone propionate. It is probable that the success of these experiments was related to the pellet form of hormone administration, although Nb rats may be particularly sensitive. When treatment was combined with estrogen, tumors appeared at a younger age. Transplants of the primary tumors were found to be autonomous. It is suggested that the intermittent variations in hormone level resulting from the rapid dissolution of the testosterone pellets and their frequent replacement favored the development of more rapid progressive tumor growth to autonomy, as outlined in a new theory of carcinogenesis (14). Concomitant treatment with estrogen, an antagonizing hormone, might be expected to exaggerate hormone fluctuation and thus accelerate malignant change. Pretreatment for some months with estrogen may make tumors of the prostate less frequent from subsequent treatment with testosterone. A unique adenocarcinoma of the prostate developed following the removal of an EP, after which the constant stimulation by endogenous androgen presumably led to malignant change. Transplants grew only in estrogenized animals and in this case behaved as a typical estrogen-dependent tumor. The biochemical parameters of this tumor model may prove to be of interest since preliminary studies by Dr. N. Bruchovsky and associates, Department of Medicine, University of Alberta, Edmonton, have shown that the tumors contain binding protein which is being characterized for both androgen and estrogen. The spontaneous prostatic adenocarcinoma (R3327) described by Dunning in a Copenhagen rat grew in females and more rapidly in males but not in castrates (21). The results presented emphasize the possible hormonal etiology of cancer of the dorsal prostate and offer a new experimental approach for a study of not only the cause but also the treatment of this condition.

ACKNOWLEDGMENTS

More recent technical assistance in these experiments given by Gabrielle Suedfeld, Noel Palmer, and Paula Naples is gratefully acknowledged.

REFERENCES


All sections H & E, × 400.

Fig. 1. Spontaneous adenocarcinoma 57 of the prostate with extensive abdominal metastases in a 56-week-old rat (bladder removed).

Fig. 2. Adenocarcinoma from rat shown in Fig. 1.

Fig. 3. Large primary adenocarcinoma 79 following TPP [3 pellets (x15)] for 84 weeks with urinary obstruction and metastases.

Fig. 4. Adenocarcinoma from rat shown in Fig. 2.

Fig. 5. Primary adenocarcinoma 60 following TPP [2 pellets (x8)] for 56 weeks confined unilaterally to coagulating gland and dorsal prostate with urinary obstruction and dilation of ureter.

Fig. 6. Primary adenocarcinoma 56 following TPP [2 pellets (x8)] for 41 weeks.

Fig. 7. Small primary adenocarcinoma 91 of dorsal prostate and right coagulating gland (R) following EP treatment for 20 weeks followed by TPP [3 pellets (x8)] for 50 weeks. Control prostate of retired breeder of same age (C). L, left.

Fig. 8. Gross vertical section of dorsal prostate shown in Fig. 7. Compact solid tumor nodule of right side of dorsal gland (R) is in contrast to uniformly porous appearance of normal left side (L).

Fig. 9. Adenocarcinoma of the dorsal prostate and adjoining normal hyperplastic gland from top of tumor nodule (right) shown in Fig. 8.

Fig. 10. Squamous carcinoma in 13th transplant of spontaneous carcinoma 9 originating in rat aged 40 weeks.

Fig. 11. Metastasis in lung from same tumor shown in Fig. 10. The metastasis is more highly differentiated with little squamous tissue.

Fig. 12. Anaplastic carcinoma in 5th transplant of adenocarcinoma 84. Primary tumor followed EP + TPP [3 pellets (x8)] for 44 weeks.

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