Influence of N-Methyl-N-Nitrosourea, Testosterone, and N-(4-Hydroxyphenyl)-all-trans-retinamide on Prostate Cancer Induction in Wistar-Unilever Rats

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ABSTRACT

The influence of chemical carcinogen, hormonal stimulation, and chronic dietary administration of the synthetic retinoid, N-(4-hydroxyphenyl)-all-trans-retinamide (4-HPR), on the induction of prostate cancer in male Wistar-Unilever rats was determined. Three different tumor induction regimens were used: (a) a single i.v. dose of 50 mg of N-methyl-N-nitrosourea (MNU) per kg body weight, followed by chronic androgen stimulation via s.c. implantation of two silastic capsules containing 40 mg testosterone each; (b) a single i.v. dose of 50 mg of MNU per kg body weight (no testosterone treatment); and (c) chronic androgen stimulation with implanted testosterone capsules (no MNU treatment). In a fourth series of animals, the incidence of spontaneous prostate tumors was determined. In groups of rats receiving neither carcinogen nor hormone stimulation. Within each series, parallel groups of animals were fed a control (vehicle-supplemented) diet or control diet supplemented with 4-HPR beginning 1 day after carcinogen administration; retinoid administration was continuous until termination of the study at 450 days. The incidence of accessory sex gland cancer in rats treated sequentially with MNU + testosterone was >60%, in comparison with cancer incidences of <20% in rats receiving MNU only and <5% in rats treated with testosterone only. No spontaneous accessory sex gland tumors were observed in rats receiving no carcinogen and no testosterone. Tumor induction in the accessory sex glands by MNU + testosterone was relatively specific for the control (vehicle-supplemented) diet or control diet supplemented with 4-HPR. This report summarizes the results of a study in which male WU rats were treated with a sequential regimen consisting of: a direct-acting chemical carcinogen + testosterone; carcinogen only; or testosterone only; to induce primary adenocarcinomas in the prostate. Tumors induced by the carcinogen + androgen regimen developed within approximately 12 to 15 months in an anatomical site that is analogous to the primary location of prostate cancers seen in humans and share a number of other characteristics with the human disease (3, 4).

INTRODUCTION

Prostate cancer is the leading cause of cancer morbidity and mortality in American men of ages 55 years and older; it has recently been estimated that ~18% of the United States male population will develop invasive prostate cancer at some time during their lifetime (1). Although 5-year survival rates for prostate cancer patients have increased dramatically over the past 30 years, age-adjusted prostate cancer death rates have also increased. During the period from 1960–1962 to 1990–1992, age-adjusted prostate cancer death rates in the United States increased nearly 30%, from 20.7 per 100,000 to 26.7 per 100,000 (1).

Because prostate cancer risk is strongly correlated with advancing age, gains in longevity throughout the 20th century have been accompanied by increases in the proportion of the American male population that is at risk for the disease. As such, the importance of prostate cancer as a public health problem has increased significantly over recent decades; this trend is likely to continue. Along with breast cancer, prostate cancer has been identified by the National Cancer Institute as an organ site of primary importance for cancer control efforts (2).

Although significant progress in cancer chemoprevention has been made in other organ sites, the prevention of prostate cancer has received only moderate attention. This is despite the fact that the prostate is an organ where effective chemopreventive regimens could have a dramatic impact on morbidity and mortality; because cancer of the prostate occurs mostly in elderly men, even a modest delay in neoplastic development achieved through pharmacological or nutritional intervention could result in a substantial reduction in the incidence of clinically detectable disease. Any lengthening of tumor latent period could delay the clinical manifestation of prostate cancer beyond the point where competing risks would intervene.

A major hindrance to the development of strategies for prostate cancer chemoprevention has been the lack of appropriate animal model systems in which such strategies can be evaluated (2). This report summarizes the results of a study in which male WU rats were treated with a sequential regimen consisting of: a direct-acting chemical carcinogen + testosterone; carcinogen only; or testosterone only; to induce primary adenocarcinomas in the prostate. Tumors induced by the carcinogen + androgen regimen developed within approximately 12 to 15 months in an anatomical site that is analogous to the primary location of prostate cancers seen in humans and share a number of other characteristics with the human disease (3, 4).

The chemopreventive activity of the synthetic retinoid, 4-HPR (fenretinide), as an inhibitor of prostate carcinogenesis was also evaluated in this study. 4-HPR was selected as a candidate chemopreventive agent for evaluation in the rat prostate cancer model system on the basis of the well-known activity of retinoids as modifiers of cell proliferation and differentiation and the demonstrated efficacy of 4-HPR as an inhibitor of carcinogenesis in other animal tumor models (5–9).

MATERIALS AND METHODS

Animals and Animal Husbandry. Before the initiation of the study, the experimental protocol was reviewed and approved by the IIT Research Institute Animal Care and Use Committee. All aspects of the program involving animal care, use, and welfare were performed in compliance with United States Department of Agriculture regulations and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Male WU (HsdCpb:WU) rats (7 to 8 weeks of age at the time of receipt) were purchased from Harlan/Sprague Dawley (Indianapolis, IN) for use in the study. Rats were housed in pairs on hardwood bedding in polycarbonate cages in a temperature-controlled room maintained on a 12-h light/dark cycle. At all times during the study, rats were permitted free access to basal diet (Teklad 4% fat rat/mouse chow; Teklad Test Diets, Madison, WI, with 3282
or without supplemental 4-HPR, and City of Chicago drinking water. Twice weekly, all rats were transferred to clean cages with fresh food, water, and bedding. Animals were observed a minimum of once daily to monitor their general health status and received a weekly clinical examination and body weight measurement.

**Experimental Design.** Four series of rats were treated with MNU + testosterone, MNU only, testosterone only, or received no carcinogen or hormone; within each series, parallel groups of rats received diet with or without supplements of 4-HPR (Table 1). Animals were distributed into experimental groups using a randomization process designed to ensure comparable mean initial body weights in all groups. Initial group size was 39–40 rats/group.

Interim kills of four to five rats per group were performed at study days 120 and 300 to permit histopathological evaluation of accessory sex gland tissues. Because no neoplastic lesions were seen in any group at the 120-day time point, animals from this time point were excluded from the histopathology data presented. Animals designated for both serial kills were excluded from the overall survival calculations; excluding animals designated for interim kills, initial group sizes used for calculation of percentage of survival ranged from 30 to 32 animals/group.

**Pretreatment.** After a quarantine period of 1 week, rats received daily i.p. injections of 8 mg of cyproterone acetate (Berlex Laboratories, Wayne, NJ) in sesame oil for 20 consecutive days. This regimen of antiandrogen administration is designed to suppress prostatic cell proliferation and induce regression of the prostatic epithelium. One day after the last cyproterone acetate dose, all rats received a single s.c. injection of testosterone propionate (100 mg/kg body weight; Sigma Chemical Co., St. Louis, MO). This testosterone dose is administered to stimulate prostatic epithelial cell proliferation and thereby maximize the sensitivity of the prostate to carcinogenic insult (3).

**Carcinogen Administration and Androgen Promotion.** Sixty h after administration of testosterone propionate, rats received a single i.v. injection of 50 mg of MNU (Ash-Stevens, Detroit, MI) per kg body weight, according to the protocol (Table 1). MNU was administered in sterile saline (pH 5.0); controls received a single i.v. injection of sterile saline (pH 5.0) only.

To evaluate the influence of chronic androgen exposure on tumorigenesis in this model system, all study animals received s.c. implants of two silastic tubes (1.2" length × 0.062" inside diameter × 0.125" outside diameter; Dow-Corning Corporation, Midland, MI), with or without crystalline testosterone (Sigma). Previous studies have demonstrated 2–3-fold increases in serum testosterone in animals receiving implants of silastic tubes containing testosterone (10). Silastic tubes were implanted immediately after MNU or saline injection and were changed at 90-day intervals throughout the study. Groups not receiving testosterone were implanted with empty silastic tubes.

**Chemopreventive Agent Administration.** Dietary administration of 4-HPR was initiated on the day after carcinogen exposure and was continued until the end of the study at 450 days after MNU. To prevent oxidative degradation, 4-HPR was admixed into test diets using a vehicle of (per kg diet);

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*Single i.v. dose of 50 mg/kg body weight, administered in sterile saline (pH 5.0).

Delivered by s.c. implanted silastic capsules containing 40 mg of testosterone propionate. Two capsules were implanted per animal and changed at intervals of 90 days.

Significantly increased (P < 0.05) versus relevant testosterone-treated comparison group (group 3 versus group 1, group 4 versus group 2, group 7 versus group 5, and group 8 versus group 6).

Table I Influence of 4-HPR on survival and body weight in WU rats receiving prostate cancer induction regimens

For 4-HPR was administered at a level of 2 mmol (782 mg) per kg diet; this level of 4-HPR was selected on the basis of the report of Ohshima et al. (11), who found that dietary administration of 4-HPR at 2 mmol per kg diet modestly suppressed the incidence of spontaneous prostate cancer in aged ACI/segHapBR rats. However, this dose level of 4-HPR resulted in an almost immediate suppression of body weight gain in the young adult rats used in the present study. As a result of this influence on body weight, the dietary level of 4-HPR was reduced to 1 mmol per kg diet at 2 weeks after the onset of exposure and was continued at the 1 mmol per kg diet level for the remainder of the 450-day study period. The 1-mmol per kg diet dose level of 4-HPR has demonstrated chemopreventive activity in the rat mammary gland (5, 7), mouse urinary bladder (6), mouse skin (8), and mouse hematopoietic system (9), among other tissues.

The concentration of 4-HPR in replicate samples of formulated test diets was measured monthly throughout the study, using analytical methods developed previously in our laboratory (12). The concentration of 4-HPR in formulated diets was 101.4 ± 8.8% (mean ± SD) of the target level.

**Necropsy and Histology.** Intercurrent deaths were necropsied immediately upon discovery; moribund animals and animals surviving until the terminal necropsy were euthanized by CO2 asphyxiation and necropsied immediately after death. At necropsy, all gross lesions were excised, and the accessory sex glands and urinary bladder were carefully removed en bloc from each rat. Individual accessory sex glands were dissected out and were fixed in 10% neutral buffered formalin. After fixation, accessory sex glands were embedded in paraffin. The seminal vesicle plus coagulating glands (anterior prostate), dorsolateral prostate, and ventral prostate were embedded separately. The dorsolateral prostate was bisected midway in the dorsal lobe, at a right angle to the prostatic urethra. Both halves of the dorsolateral prostate were embedded together, with the cut surface down. Six step sections (5 μm) each were prepared from the dorsolateral prostate and the anterior prostate plus the seminal vesicle complex; step sections were cut at intervals of 200 μm. In addition, a single section was prepared from the ventral prostate of each animal. Tissues were stained with H&E for histopathological evaluation.

**Histopathological Evaluation.** Accessory sex gland tumors were categorized on the basis of: (a) degree of malignancy; (b) site of origin; and (c) size of lesion (13). Using criteria described previously (13), proliferative epithelial lesions were classified as adenocarcinoma, carcinoma in situ, adenoma, or atypical hyperplasia; representative photomicrographs of accessory sex gland lesions induced by MNU + testosterone are provided in Figs. 1 and 2. The term adenocarcinoma was reserved for lesions that were clearly growing invasively. Atypical hyperplasia was characterized by cells that had lost cellular polarity, and that displayed clear evidence of cellular and nuclear atypia; however, these cells were confined to the acinar outline and had clearly not invaded into the surrounding tissue. Lesions classified as carcinoma in situ demonstrated all of the cellular characteristics of adenocarcinoma (more distinct atypia than in hyperplasias and/or possible penetration of the basement membrane or acinar outline) but did not display clear invasive growth. One proliferative lesion (in the anterior prostate) was distinctly expansile and compressed surrounding normal tissue. Because this lesion was clearly delineated and noninvasive, it was classified as an adenoma. Relatively few non-epithelial tumors were observed in the accessory sex glands; these lesions were classified using standard criteria.
Simultaneous with the histopathological classification of malignancy, the site of each lesion was identified. For small lesions (hyperplasia, carcinoma in situ, and small carcinomas), it was possible to define whether the site of origin was in the dorsolateral, ventral, or anterior prostate, or in the seminal vesicle. For larger carcinomas, the precise identification of a site of origin was often not possible. In such cases, tumors were scored for location in either the anterior prostate/seminal vesicle region (uncertain site of origin, but not dorsolateral prostate) or to the dorsolateral prostate region, in which instance the lesion could have originated from any of the accessory sex gland structures except the ventral prostate (dorsolateral prostate, anterior prostate, and/or seminal vesicle). The ventral prostate was never involved in large carcinomas and did not develop smaller proliferative lesions. The ampullary glands are a potential but unlikely site of origin, because no small carcinomas originating at this site have ever been observed in numerous studies conducted using this animal model.

Finally, a classification scheme was used to distinguish small lesions identified through histological evaluation of tissues from larger cancers that were macroscopically detectable (14). These macroscopic cancers may be analogous to clinically detectable prostate cancer in humans, whereas the smaller lesions may demonstrate similarity to the small histological (or latent) cancers that occur as an age-related change in the human (15). Lesions that were visible at necropsy were classified as "macroscopic." Tumors that were not detected at necropsy and were first identified during microscopic examination of tissues were classified as "microscopic." A few larger tumors that were found during microscopic examination had changed the size and shape of tissue to a degree that strongly suggested to the Study Pathologist (M. J. B.) that they were macroscopically visible but had not been identified at necropsy; these lesions were also classified as macroscopic. In the present study, carcinomas that were classified as microscopic had a maximum diameter of 3 mm or less, whereas carcinomas classified as macroscopic were 5 mm in diameter or larger (range, 5 to ~30 mm). In the present study, no accessory sex gland tumors were identified, the largest dimension of which was 4 mm, the operational boundary between microscopic and macroscopic lesions. In view of the large size of many macroscopic lesions (many exceeding 10 mm) and the lack of lesions whose size was at the delineation point between microscopic and macroscopic, a boundary of 4 mm appears to be realistic and appropriate for use in tumor classification.

Many animals demonstrated multifocal lesions, with large variations in the size of individual carcinomas; lesions in more than one accessory sex gland structure were a common finding. Because of this complexity, analysis of lesion multiplicity data was impractical; an alternate approach to data evaluation was required. Incidence data presented in Table 2 are based on the largest proliferative lesion identified in the accessory sex glands of each animal ("all accessory sex glands combined") or the largest lesion found in the dorsolateral prostate region (uncertain site of origin, as described above), the dorsolateral prostate and anterior prostate (clearly confined to one of these glands), anterior prostate/seminal vesicle region (uncertain site of origin, but not dorsolateral prostate). The number of animals with seminal vesicle lesions only (no lesions in other glands) is presented separately in Table 2. The number of rats evaluated from each group excludes animals that were designated for the 120-day interim kill and excludes animals whose tissues were lost to evaluation for reasons of cannibalism or autolysis.

Statistical Analyses. Four pairs of experimental groups were used to assess the chemopreventive efficacy of 4-HPR in the prostate. These groups were designed to evaluate the activity of the retinoid in suppressing prostate cancers that: (a) are induced by the combination of MNU + testosterone (group 2 versus group 1); (b) are induced by MNU only (group 4 versus group 3); (c) are induced by testosterone only (group 6 versus group 5); and (d) develop spontaneously in male WU rats (group 8 versus group 7). Body weight data were compared by ANOVA. Survival curves for treated and control groups were compared using life table analysis and the log-rank test (16); survival at individual time points was evaluated using \( \chi^2 \) analysis. Comparisons of prostate cancer incidence were made using Fisher's exact test (two-sided). Because no differences in survival between dietary control and 4-HPR groups were found with any carcinogen and/or hormone regimen, no corrections for mortality were required in comparisons of cancer incidence.

Chemopreventive activity was defined as a statistically significant \( P < 0.05 \) reduction in cancer incidence (incidence of all cancers, incidence of macroscopic cancers in a specific organ site, and/or incidence of microscopic cancers in a specific organ site) in a group treated with 4-HPR in comparison with the appropriate dietary control. Borderline chemopreventive activity was defined as a reduction in lesion occurrence that was significant at the 10%, but not 5%, level of confidence (0.05 < \( P < 0.10 \)).

RESULTS

Prostate Cancer Response. The highest incidences of cancers in the prostate and other accessory sex glands were seen in groups treated with MNU + testosterone (Table 2). The total incidence of adenocarcinomas in the accessory sex glands of rats receiving MNU + testosterone was 67% in dietary controls and 62% in rats receiving dietary 4-HPR. The vast majority of cancers induced by the combined regimen of MNU + testosterone appear to arise in the dorsolateral and/or anterior prostate. Approximately 50% of rats

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Footnote:

4 M. C. Bosland, unpublished observations.
treated with MNU + testosterone (45% in the dietary control group and 56% in the 4-HPR group) developed adenocarcinomas or carcinoma in situ that were confined to the dorsolateral prostate or the anterior prostate. By contrast, ~10% of rats treated with MNU + testosterone developed seminal vesicle tumors only. A reduction of borderline statistical significance in the incidence of carcinomas in the dorsolateral prostate region was achieved by 4-HPR in the MNU + testosterone group in comparison with its parallel dietary control (P = 0.096; two-sided). However, this modest protection against cancer induction was lost when the incidence of dorsolateral prostate regional cancer was combined either with cancer confined to dorsolateral or anterior prostate or with cancer of these lobes + seminal vesicle. 4-HPR had no effect on the incidence of atypical hyperplasia in the accessory sex glands of animals treated with MNU + testosterone (data not shown).

The incidences of prostatic neoplasia in rats exposed to MNU only or testosterone only were considerably lower than in animals receiving the combined MNU + testosterone regimen (Table 2). Similar to the results obtained in the comparison of groups receiving MNU + testosterone, 4-HPR conferred no protection against prostate cancer induction in animals exposed to carcinogen only (group 3 versus group 4). In fact, a statistically significant enhancement of carcinogenesis by 4-HPR was found in comparisons of the incidence of adenocarcinoma of the seminal vesicle in groups treated with MNU

![Fig. 2. All photomicrographs are of paraffin-embedded sections stained with H&E. a, microscopic adenocarcinoma of the anterior prostate. This lesion was present in five step sections of six. The lesion (A) appears to originate from the acinus and demonstrates atypical hyperplastic epithelium (H with arrowheads) in its center. The lesion is growing invasively into the surrounding tissue. Bar, 70 μm. b, microscopic adenocarcinoma of the seminal vesicle. This lesion was present in six of eight step sections. This tumor (A) is located between two adjacent folds of normal seminal vesicle epithelium (N). Bar, 70 μm. c, macroscopic adenocarcinoma that is clearly confined to the seminal vesicle. Bar, 340 μm. d, macroscopic adenocarcinoma of the dorsolateral prostate region. The exact site of origin of this tumor is not clear, because it occupies an area that includes several accessory sex gland structures. Preexisting normal glands (N) are intermingled with the glandular structures of the tumor (A).]

| Table 2 Influence of 4-HPR on percentage of incidence of accessory sex gland neoplasms in WU rats treated with MNU + testosterone, MNU only, or testosterone only (T) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Chemical carcinogen | MNU | MNU | MNU | MNU | None | None |
| Hormone treatment | None | 4-HPR | None | 4-HPR | None | 4-HPR |
| Chemopreventive agent | T | T | None | T | T | None |
| Number of animals evaluated | 33 | 34 | 34 | 28 | 33 | 34 |
| % incidence of lesions in all accessory sex glands combined | | | | | | |
| Adenocarcinoma (all) | 67 | 62 | 9 | 18 | 3 | 0 |
| Macroscopic | 24 | 12 | 3 | 7 | 3 | 0 |
| Microscopic | 42 | 50 | 6 | 11 | 0 | 0 |
| Adenocarcinoma + carcinoma in situ | 73 | 74 | 12 | 21 | 6 | 0 |
| Carcinoma in situ only | 6 | 12 | 3 | 4 | 3 | 0 |
| % incidence of lesions in dorsolateral prostate regiona | | | | | | |
| Adenocarcinoma, macroscopic | 18 | 3 3 | 3 | 0 | 3 | 0 |
| Adenocarcinoma, microscopic | 0 | 0 | 3 | 0 | 0 | 0 |
| % incidence of lesions in dorsolateral/anterior prostateb | | | | | | |
| Adenocarcinoma (≤ carcinoma in situ) | 45 | 59 | 3 | 4 | 3 | 0 |
| Macroscopic | 3 | 0 | 0 | 0 | 0 | 0 |
| Microscopic | 33 | 47 | 3 | 0 | 0 | 0 |
| Carcinoma in situ only | 9 | 12 | 0 | 4 | 3 | 0 |
| Sarcoma (undifferentiated) | 0 | 3 | 0 | 4 | 0 | 0 |
| % incidence of lesions in anterior prostate/seminal vesicle regionc | | | | | | |
| Adenocarcinoma, macroscopic | 0 | 3 | 3 | 0 | 0 | 0 |
| Sarcoma (histiocytic) | 0 | 3 | 3 | 0 | 0 | 0 |
| % incidence of lesions in seminal vesicle onlyd | | | | | | |
| Adenocarcinoma (≤ carcinoma in situ) | 12 | 9 | 0 | 18 0 | 0 | 0 |
| Macroscopic | 3 | 0 | 0 | 7 | 0 | 0 |
| Microscopic | 9 | 9 | 0 | 11 0 | 0 | 0 |
| Carcinoma in situ only | 0 | 9 | 3 | 0 | 0 | 0 |

a Originating in dorsolateral prostate, anterior prostate, or seminal vesicle.
b Confined to dorsolateral and anterior prostate; origin not clearly identifiable.
c Confined to anterior prostate and seminal vesicle; origin not clearly identifiable.
d Originating in, and clearly confined to, seminal vesicle.
e 0.05 < P < 0.10 versus appropriate dietary control group.
only \( P = 0.030 \). The importance of this observation is unclear, however, because seminal vesicle cancer in humans is exceedingly rare (17).

The incidence of accessory sex gland neoplasms in rats exposed chronically to testosterone only (without MNU; groups 5 and 6) was <5% and was not influenced by dietary exposure to 4-HPR. No spontaneous accessory sex gland tumors were found in any animal that received neither MNU nor testosterone (groups 7 and 8), regardless of exposure to the retinoid.

**Tumors in Other Sites.** Tumors in tissues other than the accessory sex glands were observed in all groups treated with MNU. Excluding the accessory sex glands, the most common sites of neoplastic development in MNU-treated rats were the kidney (17%), Zymbal's gland (17%), and lymphoma/leukemia (13%). Tumors were identified less frequently in the skin and subcutis of MNU-treated rats. Tumorigenesis in rats treated with testosterone only (no carcinogen) was limited to a single tumor in the subcutis.

The incidence of tumors in nontarget tissues was comparable in all carcinogen-treated groups; no statistically significant differences from the incidence of nontarget tumors in the MNU only group were seen in groups treated with MNU + testosterone, MNU + 4-HPR, or MNU + testosterone + 4-HPR.

**Survival.** Survival at the termination of the study was >80% in all groups that received no carcinogen. At both 300 and 450 days, comparable survival was seen in groups of saline-treated rats receiving no 4-HPR and no testosterone (group 7), 4-HPR and no testosterone (group 8), and 4-HPR plus testosterone (group 6; Table 1). Although survival at 450 days was slightly reduced in rats exposed to testosterone only (group 5), the difference between groups 5 and 7 was not significant at the 5% level.

By contrast to the high survival rates in animals receiving no carcinogen, groups treated with MNU demonstrated reduced survival beginning at ~200 days after carcinogen exposure. Mortality in groups treated with MNU resulted primarily from neoplastic development in the accessory sex glands and to a lesser degree from tumorigenesis in nontarget tissues. Between 300 and 450 days after carcinogen, MNU-treated groups fed 4-HPR (with or without testosterone; groups 2 and 4) demonstrated slightly better survival than animals receiving the same treatment without dietary supplementation with the retinoid (groups 1 and 3). These differences, however, were not significant at the 5% level of confidence (Table 1).

**DISCUSSION**

The present study was designed to characterize a rat prostate cancer model system that is suitable for in vivo evaluations of the efficacy of chemopreventive agents. The results of this study demonstrate that carcinogenesis in the accessory sex glands of WU rats is dependent both on exposure to the chemical carcinogen and on chronic hormonal stimulation by testosterone. No spontaneous accessory sex gland tumors were seen in untreated rats in the present study, and the incidence of accessory sex gland neoplasms in dietary control rats treated with either MNU only or testosterone only was <10%. By contrast, the total incidence of accessory sex gland cancer in dietary controls treated with MNU + testosterone was 67%. This incidence of malignancy in dietary controls is appropriate for in vivo chemoprevention evaluations, because it permits identification of both inhibitory and enhancing effects on prostate cancer induction.

These data also demonstrate that the primary sites of neoplastic development in this model system are the dorsolateral and anterior prostate. In dietary controls treated with MNU + testosterone, the total incidence of cancers (excluding carcinoma in situ) that were clearly confined to these prostate lobes was 36%; when in situ carcinomas are included, the total incidence of lesions clearly confined to the dorsolateral and/or anterior prostate increases to 45%. By contrast, adenocarcinoma and/or carcinoma in situ that were clearly confined only to the seminal vesicle were seen in 12% of dietary control rats treated with MNU + testosterone.

The majority of malignant lesions identified in the study were microscopic. Macroscopically visible accessory sex gland lesions were present in 24% of the dietary control group treated with MNU + testosterone. By contrast, 48% of the animals in this group demonstrated microscopic or in situ lesions but had no macroscopic evidence of neoplasia. Most of the microscopic carcinomas were present in only some of the six step sections prepared from relevant tissues. On this basis, it can be concluded that limiting evaluation of accessory sex gland tumors to microscopic lesions will result in the identification of less than half, and perhaps only one-third, of all neoplasms present. When evaluations are focused on the dorsolateral + anterior prostate, macroscopic lesions accounted for only ~10% of the total cancer incidence.

To quantify the importance of examining multiple step sections in assessing tumor incidence and chemopreventive efficacy in the pros-

![Graph](image-url)
tate, we retrospectively examined accessory sex gland slides from the MNU + testosterone dietary control group from the present study. As indicated in Fig. 3, the incidence of neoplasia diagnosed in all accessory sex glands combined or in the dorsolateral + anterior prostate increases as the number of step sections evaluated increases. A plateau is reached when five to six step sections per tissue are evaluated. These results clearly demonstrate the value of performing multiple step sections in the identification of accessory sex gland lesions in this model system and further emphasize the importance of histopathological evaluation in assessing the efficacy of novel agents for prostate cancer chemoprevention.

Dietary administration of 4-HPR conferred no protection against the induction of prostate cancers or other accessory sex gland tumors in WU rats by MNU + testosterone or by MNU alone. Prostate cancer incidence in rats exposed to testosterone only (no MNU) was too low to permit adequate evaluations of anticarcinogenic activity. On the basis of these results, it is concluded that 4-HPR is not an effective chemopreventive agent when administered at its maximum tolerated dose in the WU rat prostate cancer model system.

The chemoprevention data from the present study are in general agreement with the results reported by Slayter et al. (18) and Lucia et al. (19), who examined the influence of 4-HPR on the induction of accessory sex gland lesions by MNU + testosterone propionate in Lobund-Wistar rats using extensive histopathological evaluation of accessory sex gland tissues. The rat strain, tumor induction regimen, and approach to histopathological classification used in the present study differ from those used by Slayter et al. (18) and Lucia et al. (19); however, the results of all three studies are generally comparable. Neither Slayter et al. (18) nor Lucia et al. (19) found that groups fed 4-HPR demonstrated overall reductions in prostate cancer incidence. Lucia et al. (18) did report an inhibition by 4-HPR of the incidence of invasive carcinoma in the anterior prostate in animals treated with MNU + a low dose of androgen. However, Lucia et al. (19) and Slayter et al. (18) both reported that 4-HPR increased the incidence of invasive carcinoma of the anterior prostate in rats treated with higher doses of MNU + androgen. Neither reported any significant effect of 4-HPR on the incidence or severity of cancer of the dorsolateral prostate, a tissue with embryological homology to the human prostate (20), and the primary site of cancer induction by MNU + testosterone in our study.

The data from these three studies differ from those presented by Pollard et al. (21), who reported a significant inhibition by dietary 4-HPR of the induction of accessory sex gland tumors in Lobund-Wistar rats by MNU + testosterone propionate. A significant difference in our approaches to chemoprevention efficacy evaluation, however, is that the tumor incidence data reported by Pollard et al. (21) were limited to lesions that were detectable on the basis of palpation and gross pathology. No histopathology was reported, and it must be assumed that no microscopic tumors were included in their results. By contrast, the results of the present study and the data reported by Slayter et al. (18) and Lucia et al. (19) include both macroscopic and microscopic cancers that were identified by step sectioning and histopathological evaluation of accessory sex gland tissues.

It is also interesting to note that Slayter et al. (18) and Lucia et al. (19), as well as Cohen et al. (22) and Tamano et al. (23), have reported that the majority of accessory sex gland tumors induced by MNU + testosterone propionate in the Lobund-Wistar rat arise in the seminal vesicle rather than in the prostate. On this basis, it is logical to conclude that the majority of the gross lesions identified by Pollard et al. (21) in their chemoprevention study with 4-HPR were tumors of the seminal vesicle rather than of the prostate. In view of the extremely rare occurrence of seminal vesicle tumors in humans (17), it would appear that the WU rat model used in the present study is more appropriate for prostate cancer chemoprevention studies than is the Lobund-Wistar rat model used by Pollard et al. (21).

It should also be noted that Oshishina et al. (11) reported that dietary administration of 4-HPR resulted in a small reduction in the incidence of spontaneous prostate tumors in aged ACI/segHapBR rats. In that study, 4-HPR was administered at a level of 2 mmol per kg diet to retired breeders for up to 54 weeks; 4-HPR reduced prostate cancer incidence from 43% in dietary controls to 28%, although this reduction did not achieve statistical significance. It appears that spontaneous tumorigenesis in the accessory sex glands of the aged ACI/segHapBR rat is a fundamentally different process than the induction of accessory sex gland cancers by MNU + androgen in both WU and Lobund Wistar rats; the primary site of tumor development in the aged ACI/segHapBR rat is the ventral prostate (24, 25), a prostate lobe in which neoplastic development is extremely infrequent in both WU and Lobund-Wistar rats.

4-HPR also reduced tumor incidence and mass in a murine prostate cancer model in which fetal urogenital sinus tissues are infected with viral oncogenes and then reconstituted in vivo (26). The murine prostate cancer reconstitution model is characterized by an extremely rapid rate of neoplastic development (85% tumor incidence in 7 weeks) and a high degree of anaplasia (26). As such, this model may more closely simulate advanced human prostate cancer than the process of neoplastic development involving a long latent period. Of potential relevance in this regard is the report that 4-HPR has therapeutic activity in a transplantable model of rat prostate cancer (27).

The results of the present study demonstrate the utility of the prostatic adenocarcinoma induced in WU rats by MNU + testosterone as a model for chemopreventive efficacy evaluations. Furthermore, this study demonstrates the need for extensive standardized histopathological evaluation in such assessments. Although 4-HPR had no chemopreventive activity in the present study, the retinoid has been shown to confer protection against cancer induction in a number of other in vivo carcinogenesis model systems (5–9). On this basis, 4-HPR does offer significant promise as a candidate agent for the prevention of human cancer.

REFERENCES

PROSTATE CANCER INDUCTION IN WISTAR-UNILEVER RATS


Influence of \( N \)-Methyl-\( N \)-Nitrosourea, Testosterone, and \( N \) -\((4\text{-Hydroxyphenyl})\text{-all- trans-retinamide}\) on Prostate Cancer Induction in Wistar-Unilever Rats


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