Treatment of Prostate Cancer in the Rat with the Synthetic Retinoid Fenretinide

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Abstract

N-4-Hydroxyphenylretinamide (fenretinide or 4HPR), a derivative of retinoic acid, has been demonstrated to decrease the development of prostate cancer in a rat carcinogenesis model. This study was undertaken to determine if 4HPR is an effective agent for the treatment of established prostate cancer. In vitro, 4HPR was cytotoxic to rat and human prostate cancer cells as well as endothelial cells. Utilizing three different angiogenesis inhibition assays, it was demonstrated that 4HPR inhibited angiogenesis as well as endothelial cell motility and tubule formation. In vivo, 4HPR inhibited prostate cancer growth in a significant manner. These findings suggest that 4HPR may be a potent inhibitor of early prostate cancer growth.

Introduction

Prostate cancer is now the most common cancer diagnosed in American men, with an estimated 122,000 new cases in 1991 (1). Unfortunately, prostate cancer which has escaped the confines of the prostate gland remains incurable in the majority of patients. Through the use of the new biological markers such as prostate-specific antigen, it may now be possible to identify prostate cancer at an early stage and intervene in the process of cancer growth and progression. The potential for chemical intervention (chemoprevention or chemotherapy) as a means of halting or delaying the process of prostate carcinogenesis has yet to be explored fully.

Vitamin A and its analogues (retinoids) are currently being actively studied as chemopreventive as well as therapeutic agents (2-4). Within the nucleus, retinoids act as transcriptional regulators, generally inhibiting growth and promoting epithelial cell differentiation. Clinical research utilizing retinoids, the analogues of vitamin A, has demonstrated activity, both in vitro and in vivo, in modulating cell growth, motility, and maturation (2-4). The retinoids have been demonstrated to suppress chemical carcinogenesis in the mammary gland, bladder, skin, and liver (2-4).

4HPR is a synthetic retinoid which has been demonstrated to be effective and relatively nontoxic in preclinical experiments and early clinical trials (5-7). It has been demonstrated to inhibit the formation of mammary tumors both in vitro and in vivo (5). Although the exact mechanism of action of 4HPR has not been fully elucidated, it has been demonstrated to inhibit the process of carcinogenesis in the rat prostate (8).

The majority of men over the age of 50 years have histologically identifiable but not clinically evident prostate cancer (9). It may be clinically useful to identify nontoxic agents which are effective in treating small volumes of prostate cancer by intervening in the tumor growth and promotion process. One such strategy for accomplishing this growth inhibition is to inhibit the neovascularization of tumors, and the retinoids, among a variety of their actions, have been demonstrated to inhibit angiogenesis (10). This study was undertaken to determine if 4HPR is an effective agent for the treatment of early prostate cancer.

Materials and Methods

Materials

4HPR was provided by Dr. Fred Minn of R. W. Johnson Pharmaceutical Research Institute (Spring House, PA). It was prepared as a sterile stock solution of 1 mg/ml in 95% ethanol. Hydrocortisone succinate, all-trans-retinoic acid, 13-cis-retinoic acid, and gold were obtained from Sigma Chemical Co. (St. Louis, MO) and prepared as sterile stock solutions of 1 mg/ml in 95% ethanol. Matrigel was obtained from Collaborative Research, Inc. (Bedford, MA).

Cell Culture

The MLL subtype of the Dunning R-3327 rat prostate adenocarcinoma line and the human prostate cancer cell line PC-3 were obtained from Dr. John Isaacs (Johns Hopkins University, Baltimore, MD). Cells were grown and maintained in RPMI 1640 containing 10% fetal bovine serum and 1% penicillin/streptomycin. CPAE bovine pulmonary artery endothelial cells (ATCC CCL 209) were obtained from the American Type Culture Collection (Rockville, MD) and maintained in Eagle's minimum essential medium with Earle's balanced salt solution supplemented with 20% fetal bovine serum. Stock 4HPR, all-trans-retinoic acid, and 13-cis-retinoic acid were added to the cell culture medium to reach the reported concentrations.

Cell Growth and Viability Assays

MLL, PC-3, and CPAE cells were seeded at a concentration of 1 × 10^6 cells/25-ml flask. Drugs were added in appropriate concentrations at time zero, and flask were compared to control flasks at hour 48. Cells in the media were combined with adherent cells after their release with trypsin. Trypan blue exclusivity for cell viability was measured by adding 0.4% in 0.9% saline to a 50% dilution, and cell counts were performed in duplicate using a hemocytometer as previously described (11). Three flasks were used for each time point.

Angiogenesis Assays

Motility Assay. This assay was based on the ability of endothelial cells to clear colloidal gold particles from the substratum as described previously (12). CPAE cells (2000) were placed in 35-mm tissue culture dishes containing 2 ml of growth medium with or without drug present. After 24 h, the phagokinetic tracks were visualized by observing the cells with a Zeiss Axiovert inverted microscope at ×200 magnification. Images of 100 cells from the microscopic field were morphometrically analyzed using JAVA video analysis software (Jandel Scientific, Corte Madera, CA), and the distances cells traveled (μm/24 h) were obtained.

CAM Assay. The ability of 4HPR to inhibit angiogenesis was determined using a modification of the CAM assay as described by Crum et al. (13). Chicken embryos were obtained from Townline Poultry Farm (Zeeeland, MI) and placed in an incubator (1% CO2-air) at 37°C. On day 3, the eggs were cracked and placed in Petri dishes. Ten μl of 4HPR at the appropriate concentration were dissolved in 0.45% methylcellulose and air dried. These disks were then placed on the outer third of the day 3 embryos. The zone around the

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3 The abbreviations used are: 4HPR, fenretinide (N-4-hydroxyphenylretinamide); CAM, chicken chorioallantoic membrane; MLL, Mat-LyLu.
Animals

Male Copenhagen rats (200 g) were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Methoxyflurane (Pittman-Moore, Washington Crossing, NJ) was used as an inhalation anesthetic for injections and surgical procedures.

Experimental Treatment

Utilizing an Animal Investigation Committee-approved protocol, animals were given s.c. injections of 250,000 MLL cells in the right flank on day 0. 4HPR was dissolved in 100% ethanol and then diluted in water to the appropriate concentration. This procedure resulted in a 1% alcohol drinking water solution with a fine suspension of 4HPR. The drinking water was prepared for each dose, and drug was not noted to precipitate out in this time period. Animals drank similar amounts of water in all groups, and the 1% ethanol solution did not affect tumor growth in a separate control group (data not shown). On day 14, animals were euthanized with methoxyflurane, and cardiac puncture was performed to obtain a blood sample. Tumors were excised and weighed. Animal weights were recorded on days 0 and 14 after tumor removal. Experimental groups consisted of five animals, and each dose of 4HPR was tested in two different experimental groups at different times.

Statistics

Statistical analyses were performed using Student’s t-test as well as ANOVA utilizing Statgraphics v5 (STSC, Inc., Rockville, MD).

Results

The effect of 4HPR on the growth of the anaplastic, androgen-independent MLL, the human prostate adenocarcinoma cell line PC-3, and the endothelial cell line CPAE in vitro was investigated (see Fig. 1). In doses of 25 nM to 2.5 μM, 4HPR had no effect on the growth of MLL cells; however, a sharp dose-response curve was demonstrated between 2.5 and 25 μM. The effect of 4HPR on the human prostate cancer cell line PC-3 and the bovine endothelial cell line CPAE was also investigated (see Fig. 1). The PC-3 cells and the CPAE cells were slightly more sensitive to the retinoid, demonstrating a dose-response curve between 250 nM and 10 μM. All-trans-retinoic acid and 13-cis-retinoic acid were less cytotoxic to the cell lines than 4HPR by approximately one log dose (data not shown). These experiments demonstrated that 4HPR could be cytotoxic to prostate cancer cells, but only at relatively high concentrations.

Earlier reports have suggested that the retinoids appear to inhibit angiogenesis, possibly by inhibiting endothelial cell motility (10, 12). We utilized three different angiogenesis inhibition assays to determine the effect of 4HPR on endothelial cell motility and angiogenesis (see Table 1). In the CAM assay, 4HPR inhibited CAM blood vessel formation in doses as low as 25 nM. This inhibition was not enhanced by the presence of hydrocortisone (data not shown). 4HPR (25 nM) also inhibited the formation of endothelial tubules, an effect which was even more dramatic at concentrations above 250 nM. Significant inhibition of endothelial cell motility was demonstrated at 4HPR concentrations above 250 nM. Taken together, the results of these three assays suggest that 4HPR could potentially function as an inhibitor of neoangiogenesis during tumor growth.

Discussion

Hormonal therapy is the first line of treatment for prostate cancer, and a number of ablative therapies have been developed based upon blocking the actions of androgens (15). In spite of continued refinements in the hormonal therapies, there has been a lack of improvement in the disease-free interval or the overall 5-year survival of patients with prostatic carcinoma as patients eventually fail androgen manipulation (15). The need for new intervention strategies is clear, and it is especially enticing to consider the treatment of prostate cancer at very early stages with either a chemopreventive or early, nontoxic chemotherapeutic strategy.

Sporn et al. have recently demonstrated that 4HPR could be used to prevent the development of primary prostate cancer in the Lobund-Wistar rat model developed by Pollard (8). Animals were fed 4HPR in
4HPR has demonstrated limited toxicity in animal models. Mild mucocutaneous dryness, mild hair loss, and midnight blindness have been demonstrated (6, 7). Rotmensz et al. (18) performed a Phase II trial of 4HPR for the chemoprevention of recurrent breast cancer in women and found that the drug was well tolerated, with a low toxicity profile.

The need for new strategies to treat and/or prevent prostate cancer is clear. 4HPR has been demonstrated to inhibit the carcinogenic process as well as prostate tumor growth in the rat, with little demonstrable toxicity. Before large chemoprevention trials can be undertaken for prostate cancer, the safety of agents in the older male population at risk for prostate cancer must be assessed. 4HPR appears to be a promising agent for prostate cancer chemoprevention as well as early prostate cancer treatment.

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References

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