Prostate Cancer and Prostatic Diseases (2000) 3, 275–279
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1365–7852/00 $15.00
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Polyamine depletion therapy in prostate cancer

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The prostate gland has among the highest level of polyamines in the body and prostate carcinomas have even greater elevated polyamine levels. These ubiquitous molecules synthesized by prostate epithelium are involved in many biochemical processes including cellular proliferation, cell cycle regulation, and protein synthesis. These properties have made polyamines a potential target for therapeutic intervention in diseases of excessive cell proliferation such as cancer. However, attempts to limit tumor growth by inhibition of polyamine synthesis have not been very successful since cells have the capacity to take up polyamines from the bloodstream. We report here studies utilizing polyamine depletion by means of a combination of blockade of polyamine synthesis with DFMO (α-difluoromethylornithine), an inhibitor of ornithine decarboxylase, the rate limiting enzyme in the polyamine synthetic pathway, and ORI 1202, a novel inhibitor of polyamine transport into the cell. This cytostatic combination, even in the presence of excess extracellular polyamines, significantly slowed the growth of the human tumor cell line PC-3 grown in tissue culture with an EC_{50} in the μM range. Other prostate cell lines were similarly growth inhibited including LNCaP.FGC and DU145. Growth of the PC-3 tumor cell line as a xenograft in nude mice was also slowed significantly by this combination of compounds. Polyamine levels in the tumor were lowered from control tumor levels. This combination therapy could provide an effective and potentially non-toxic therapy for prostate tumors. *Prostate Cancer and Prostatic Diseases* (2000) **3,** 275–279.

Keywords: prostate cancer; polyamines; novel therapy

Introduction

The polyamines putrescine, spermidine, and spermine play a critical role in the growth of both normal and neoplastic cells. These ubiquitous molecules interact with DNA, RNA, proteins, and lipids. The pathways of polyamine metabolism and roles played by these molcules in cellular growth have been described in a number of reviews.[1–3]

It has long been known that prostate tissue has a very high level of polyamines. The polyamines are made in the epithelial cells and are secreted into seminal fluid.[4] Prostate tumors have levels of polyamines that are significantly elevated from the already high levels of normal prostate tissue.[4]

A number of approaches have been attempted to treat prostate tumors via polyamine pathways, including the use of the polyamine synthesis inhibitor DFMO (α-difluoromethylornithine), which inhibits ornithine decarboxylase (ODC) the rate limiting enzyme in biosynthesis, and inhibitors of other parts of the polyamine metabolic pathway such as AdoMetDC (S-adenosylmethionine) and PAO (polyamine oxidase) inhibitors.[5,6] These approaches have been largely unsuccessful. One possible explanation for the failure of these approaches is that polyamines are readily available from the circulation, via diet and production by intestinal microflora,[4,7] and that cells increase their uptake of these polyamines to compensate for drug induced low levels. The studies reported here combine the blockade of polyamine synthesis, by blocking ODC, with a blockade of the polyamine transport mechanism.

Materials and methods

Compounds

ORI 1202 (N^1-spermine-L-lysinyl amide) was synthesized as previously reported.[8] The ornithine decarboxylase (ODC) inhibitor, α-difluoromethylornithine (DFMO) was obtained from Marion Merrell Dow (Cinncinati, OH).

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Received 15 June 2000; revised 7 August 2000; accepted 14 August 2000

Cell lines and culture conditions

The human prostate cell lines were all obtained from the American Type Culture Collection (Rockville, MD). LNCaP.FGC is a well-differentiated androgen responsive, prostate specific antigen expressing, prostate carcinoma.[9] DU145 is a moderately differentiated brain metastasis of a prostate carcinoma,[10] and PC-3 is a poorly differentiated bone metastasis of a prostate carcinoma.[11] All lines were cultured in the recommended media (Mediatech, Herndon, VA), serum (Gibco, BRL, Gaithersburg, MD) supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin and 2 mM L-glutamine (BioWhittaker, Walkersville, MD). Serum amine oxidase activity was inhibited by inclusion of 1 mM aminoguanidine (Sigma, St Louis, MO). Spermidine (1 μM, Sigma) was added to cell cultures as a source of external polyamines.

Cell growth inhibition assay

Cells were plated in 96-well plates such that they would remain in log growth for the duration of the assay. The day after plating, compounds were added, and cell growth measured after 6 days by MTS/PMS dye assay (Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation Assay; Promega, Madison, Wl). The EC_{50} values represent the concentration of ORI 1202 that resulted in 50% of maximum growth inhibition achievable in the presence of DFMO and ORI 1202.

Polyamine analysis

Cultured cells or tumors were harvested, washed, and lysed in 0.4 M perchloric acid. The HPLC method for the fluorometric detection of polyamines from the extracts is based on the procedure of Kabra *et al*.[12] Standard curves were derived for each of the polyamines and were shown to be linear with a detection limit of 0.8 pmol.

Animal studies

The PC-3 cell line was grown as a xenograft in nude mice. Tumor was implanted subcutaneously by trocar with fragments harvested from sc growing tumors maintained in nude mice hosts. Animals were pair-matched in treatment and control groups when tumors reached approximately 55 mg in size (11 days post inoculation). Groups contained 12 mice which were ear tagged and followed individually throughout the experiment. DFMO was added to the drinking water, *ad libitum*, throughout the study at 1 or 3%. ORI 1202 was given as a sterile solution in water intraperitoneally, three times daily at 30 or 45 mg/kg/dose, on a 5-day per week schedule. The experiment was terminated when control tumors reached a size of 1 g. Upon termination, all mice were weighed, killed, and their tumors excised and weighed.

Statistical analysis

Analysis of variance (ANOVA) was performed on the data from the tumor xenograft study indicating significant differences between at least two of the samples ($F = 3.952$, $P < 0.001$). A Tukey test was then used to compare each treatment sub-group to the control group.

Results

Initial studies looked at the effect of polyamine depletion in several prostate tumor cell lines. In the studies shown in Figure 1, the cell lines were grown in the presence of the indicated concentrations of inhibitors for a period of 6 days. A source of extracellular polyamine was provided by growing the tumors with spermidine (1μM) in the medium. Growth of each of the cell lines was inhibited by the combination of ORI 1202 plus DFMO while neither compound alone was able to block cell growth. EC_{50} values of 5.3, 5.0, 2.6 μM were estimated for cell lines, PC-3 (Figure 1a), DU145 (Figure 1b), and LNCaP.FGC (Figure 1c), respectively. While DFMO alone was ineffective in blocking cell growth, it is worth noting (data not shown) that in the absence of external polyamine, DFMO blocks growth with EC_{50} values of approximately 100 μM.

The level of polyamines was evaluated in the PC-3 cell line, cultured in the presence of exogenous spermidine, following 1 week with or without ORI 1202, DFMO, or both. Table 1 shows that in the PC-3 cell line DFMO alone lowered the level of putrescine but had little effect on the spermidine or spermine levels. ORI 1202 by itself had very little effect on polyamine levels, but in combination with DFMO, led to major reductions in putrescine and spermidine while the level of spermine was not changed.

Based on the demonstration that polyamine depletion therapy was cytostatic for human prostate cell lines in culture, an experiment was conducted to determine the effect of this treatment on one of the prostate cell lines grown as a xenograft in nude mice. Mice were inoculated with PC-3 cells and the tumor established. After 11 days, treatment was initiated with ORI 1202 (ip at 30 and 45 mg/kg/tid 5 days/wk), DFMO (1 or 3% in drinking water, *ad libitum*), or dual compound treatment. When control tumors reached 1 g at 14 days after the start of treatment the experiment was terminated and tumor weights determined. The results are shown in Table 2. Treatment of mice with either ORI 1202 alone or DFMO alone had no significant growth inhibitory activity in this experiment ($P > 0.05$). The lower dose level of ORI 1202 showed significant inhibition of tumor growth, 40%, when combined with DFMO at 3% ($q = 4.625$, $P < 0.05$). Combining the two compounds at the high dose of ORI 1202 gave the maximum growth inhibition: 46% inhibition in combination with 1% DFMO ($q = 5.275$, $P < 0.05$) and 61% growth inhibition with 3% DFMO ($q = 7.344$, $P < 0.05$). There were no overt indications of toxicity to animals in any of the groups and only small changes were observed in body weight.

In order to determine whether the treatment was acting *in vivo* to decrease polyamines in the tumor, samples of tumor from the necropsies were assessed for polyamine levels. The results are shown in Table 3. Treatment of mice with DFMO led to a 33% reduction of the level of putrescine in the tumor. Treatment of tumor bearing mice with the combination of ORI 1202 and DFMO led to a 64% reduction of putrescine in the tumor. Spermidine

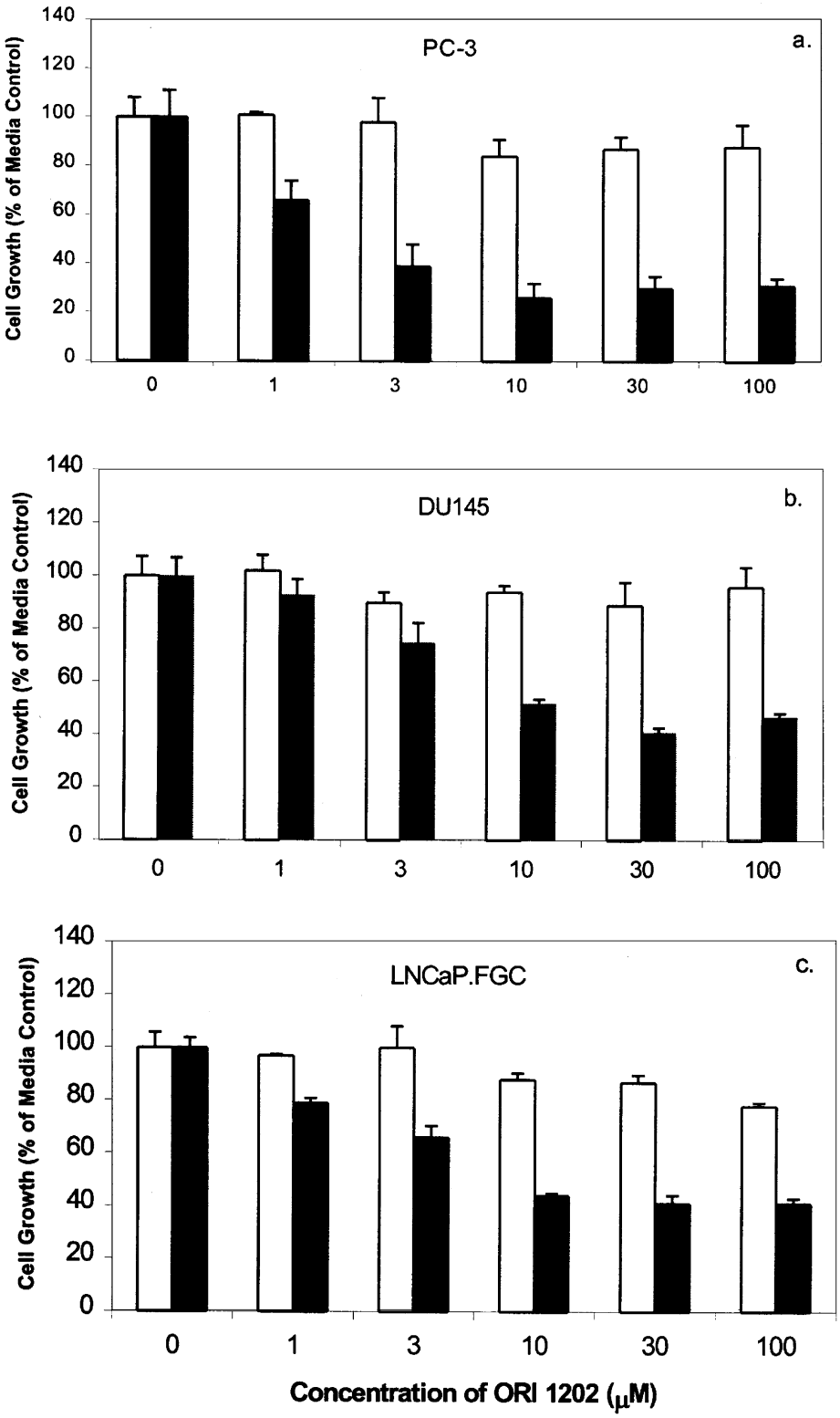

Figure 1 *Growth inhibition of cell lines in culture. The cell lines PC-3 (a), DU145 (b), and LNCaP.FGC (c) were incubated for 6 days in culture with ORI 1202 alone (open bars) at the indicated concentrations, or in combination with DFMO (solid bars) at 5 mM (PC-3 and LNCaP.FCG) or 1 mM (DU145). No growth inhibition was seen with DFMO alone. Cell growth was determined by the MTS/PMS assay from triplicate wells. Data shown are mean ± standard deviation. Included on all cultures were 1 mM aminoguanidine and 1 μM spermidine.*

Table 1 Effect of polyamine depletion therapy on polyamine levels in the PC-3 cell line grown in culture

Treatment	Putrescine	Spermidine (nmoles/10^6 cells)	Spermine
Control	1.63	1.74	2.44
DFMO (1 mM)	0.56	1.94	3.15
ORI 1202 (100 μM)	3.00	1.48	2.96
ORI 1202 (100 μM) DFMO (1 mM)	0.18	0.46	4.29

levels decreased slightly while no change was seen in the levels of spermine.

Discussion

This paper reports on the use of polyamine depletion therapy in xenograft models of prostate cancer. Attempts have been made in the past to use polyamine depletion in prostate cancer treatment with only modest success. The compounds tested acted by blocking polyamine synthesis which is required for cell growth. However, prostate cells have an active polyamine transport mechanism, which is upregulated when polyamine levels fall. Polyamines are readily available from the bloodstream, derived from food and the native bacteria of the gastrointestinal tract. In the studies reported here, we have combined a block of polyamine synthesis using DFMO, an irreversible inhibitor of ornithine decarboxylase, the rate limiting enzyme in polyamine biosynthesis, with ORI 1202, a competitive inhibitor of polyamine transport. This combination of agents resulted in a more complete depletion of putrescine and spermidine than was achieved with DFMO alone. Growth of human prostate tumor cell lines in culture was slowed with this combination treatment, as was the growth of one of these cell lines, PC-3, in nude mice. Neither agent by itself was as effective as the combination.

The polyamine depletion approach to therapy for human prostate cancer cells generated cytostasis in cultured cells rather than cytotoxicity. Removal of compounds from treated cultures by washing the cells resulted in the re-establishment of cell growth and high cell viability was observed in the cultures (data not shown). The lack of toxicity to the cultured cells correlated with a lack of toxicity in treated animals.

Table 3 Effect of polyamine depletion therapy for 14 days on polyamine levels in prostate tumors grown in nude mice

Group	n	Dose	Putrescine	Spermidine	Spermine
			nmoles/gram (mean ± s.e.m.)		
Control	3	PBS	393 ± 40	1229 ± 85	953 ± 93
DFMO	3	3%	262 ± 29	1302 ± 29	1191 ± 105
ORI-1202	3	45 mg/kg	413 ± 11	1171 ± 95	950 ± 28
ORI-1202 DFMO	3	45 mg/kg 3%	142 ± 39	946 ± 65	942 ± 105

In both the tissue culture and animal experiments treatment with DFMO and ORI 1202 showed differential effects on the three primary polyamines with the greatest depletion in putrescine, followed by spermidine and relatively minor changes in the level of spermine. The roles of the individual polyamines are not clearly delineated, but it is clear that blockade of cell growth does not require that spermine be depleted. It has been shown that spermine normally has a very slow turnover.[1,5] In addition, inhibitors of spermine synthetase have been used to suggest that spermine plays a minor role in cell growth. It has been suggested[5] that the low toxicity might be due to the maintenance of spermine levels. When spermine is reduced along with putrescine and spermidine, cellular cytotoxicity is seen.

Conclusion

We have examined the effect of polyamine depletion in prostate tumors with a combination treatment which blocks both synthesis of new polyamines with the ODC inhibitor DFMO and uptake of polyamines from the blood with the polyamine transport inhibitor ORI 1202. This combination treatment blocked growth of human prostate tumor cell lines in tissue culture as well as slowing the growth of human prostate tumor in a nude mouse xenograft model. This treatment induced a cytostatic effect on tumor cells rather than cytotoxicity and produced no toxic effects. The level of polamines was shown to be significantly reduced in treated tissue culture and xenografted tumors. The dual treatment was much more efficaious than either agent alone and could provide

Table 2 Effect of polyamine depletion therapy on growth of the human PC-3 prostate carcinoma in nude mice after 14 days of treatment

Group	n	Dose	Average body wt. change	Final tumor wt. (mg) mean ± s.e.m.	% Tumor growth inhibition
Control	12	PBS	+0.28%	1018 ± 130	—
ORI-1202	12	30 mg/kg	− 0.95%	783 ± 53	25
ORI-1202	12	45 mg/kg	+0.57%	644 ± 61	39
DFMO	12	1%	+0.52%	762 ± 93	27
DFMO	12	3%	+0.90%	673 ± 86	36
ORI-1202 DFMO	12	30 mg/kg 1%	− 3.35%	695 ± 91	34
ORI-1202 DFMO	12	30 mg/kg 3%	− 3.86%	630 ± 72*	40
ORI-1202 DFMO	12	45 mg/kg 1%	− 2.60%	576 ± 84*	46
ORI-1202 DFMO	12	45 mg/kg 3%	− 5.11%	402 ± 55*	61

*Significant at $P < 0.05$.

a new modality of treatment to add to our clinical armamentarium for prostate disease.

References

1 Pegg AE. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res* 1988; **48**: 759–774.
2 Janne J, Alhonen L, Leinonen P. Polyamines: from molecular biology to clinical applications. *Ann Med* 1991; **23**: 241–259.
3 Heby O. Role of polyamines in growth and differentiation. *Differentiation* 1991; **19**: 1–20.
4 Heston WDW. Prostatic polyamines and polyamine targeting as a new approach to therapy of prostatic cancer. *Cancer Surveys, Prostate Cancer* 1991; **11**: 217–238.
5 Martin LJ, Pegg AE. Polyamines as targets for therapeutic intervention. *Ann Rev Pharmacol Toxicol* 1995; **35**: 55–91.
6 Schecter PJ, Barlow JL, Sjoerdsma A. Clinical aspects of inhibition of ornithine decarboxylase with emphasis on therapeutic trials of eflornithine (DFMO) in cancer and protozoan diseases. In: McCann PP, Pegg AE, Sjoerdsma (eds). *Inhibition of Polyamine Metabolism. Biological Significance and Basis for New Therapies*, Academic Press: Orlando, 1987, pp 245–364.
7 Sarhan S, Knodgen B, Seiler N. The gastrointestinal tract as polyamine source for tumor growth. *Anticancer Res* 1989; **9**: 215–224.
8 Weeks RS *et al.* Novel lysine–spermine conjugate inhibits polyamine transport and inhibits cell growth when given with DFMO. *Experimental Cell Research* (in press).
9 Horoszewicz IS *et al.* LNCaP model of human prostate cancer. *Cancer Res* 1983; **43**: 1809–1818.
10 Mickey DD *et al.* Heterotransplantation of a human prostate adenocarcinoma cell line in nude mice. *Cancer Res* 1978; **37**: 4049–4058.
11 Kaighn NH *et al.* Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Invest Urol* 1979; **17**: 16–23.
12 Kabra PM *et al.* Solid-phase extraction and determination of dansyl derivatives of unconjugated and acetylated polyamines by reversed-phase liquid chromatorgraphy. *J Chromatog* 1986; **380**: 19–32.