Tumor-targeted Apoptosis by a Novel Spermine Analogue, 1,12-Diaziridinyl-4,9-diazadodecane, Results in Therapeutic Efficacy and Enhanced Radiosensitivity of Human Prostate Cancer

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ABSTRACT

Interference with polyamine transport and biosynthesis has emerged as an important anticancer strategy involving polyamine analogues and specific inhibitors of key biosynthetic enzymes. Because the prostate gland has a high polyamine content, by using the polyamine transporter for selective uptake into cancer cells, alkylating polyamines are likely to be highly effective against prostatic tumors. We have recently synthesized a novel class of spermine analogues, the lead compound of which has efficacy against human cancer cells (P. S. Callery et al., U. S. patent, 5,613,239, Issued March 17, 1997.). In this study, to investigate the potential therapeutic efficacy of the lead spermine analogue 1,12-diaziridinyl-4,9-diazadodecane (BIS), against advanced prostate cancer, we examined the in vitro effect and in vivo efficacy of the compound in two androgen-independent human prostate cancer cell lines, PC-3 and DU-145. BIS exhibited a dose-dependent cytotoxic effect against prostate cancer cells via induction of apoptosis. Treatment of cells with BIS (1 μM) for 24 h resulted in a significant induction of apoptosis (24%). Exposure of BIS-treated PC-3 prostate cancer cells to γ-irradiation resulted in a significant increase in the number of cells undergoing apoptosis and a subsequent decrease in the IC50. Furthermore, BIS treatment led to a significant enhancement of loss of clonogenic survival in irradiated prostate cancer cells (both PC-3 and DU-145). In vivo efficacy trials demonstrated a significant antitumor effect of BIS against both PC-3 and DU-145 tumor xenografts in severe combined immunodeficient mice in a dose-dependent pattern at maximally tolerated doses. Terminal transferase end-labeling analysis indicated that BIS-mediated tumor regression in vivo occurs via induction of apoptosis among prostatic tumor cells. These results suggest that the novel spermine analogue BIS: (a) has a potent antitumor effect against prostatic tumors via induction of apoptosis; and (b) increases the radiosensitivity of human prostate cancer cells by decreasing the apoptotic threshold to radiation. This study may have important clinical implications for the manipulation of this antitumor activity of the polyamine analogue for the optimization of the therapeutic efficacy of radiation in patients with advanced prostate cancer.

INTRODUCTION

Prostate cancer is a major contributor to cancer mortality among males in the United States (1). While radical prostatectomy is considered curative for localized disease, no treatment for metastatic prostate cancer is available that effectively increases survival (2). Prostate cancer mortality results from metastasis to the bone and lymph nodes and progression to androgen-independent disease. Androgen ablation monotherapy, the major therapeutic modality for advanced prostate cancer, is rarely curative because it is targeted exclusively against the androgen-dependent prostate cancer cell populations, whereas the androgen-independent cells survive this therapy and continue to grow (2). Sporadic attempts at combination regimes of hormonal ablation and chemotherapy have failed to provide clinically convincing evidence of significant success in therapeutic response (3).

Radiation therapy has been proven to be a relatively effective treatment for localized prostate cancer, with local tumor control and improvement of patient survival (4). Therapeutic synergy between radiotherapy and androgen ablation in patients with advanced metastatic prostate cancer has been documented with promising effects on the clinical outcome (5). The cellular response to lethal doses of ionizing irradiation, by undergoing apoptotic cell death, has been recognized as a primary determinant of the radiosensitivity of various tumor cells including human prostate cancer cells (6, 7). Apoptosis has emerged as a significant therapeutic target for the effective elimination of prostate cancer cells in both androgen-dependent and androgen-independent tumors, in response to androgen deprivation and chemotherapeutic drugs or radiation, respectively (8). Identification of molecules that will potentiate tumor radiosensitivity will enable the optimization of radiation therapy for the treatment of advanced prostate cancer by enhancing the therapeutic response to radiation while circumventing the problem of systemic toxicity associated with the molecule.

Polyamines represent a potentially valuable arena for the development of therapeutic strategies targeted at specific DNA sites in the treatment of cancer in light of the ubiquitous distribution of these native DNA binding compounds in mammalian cells and their central involvement in growth regulation of normal and malignant cells (9, 10). Several polyamine analogues that interfere with polyamine function and metabolism have been synthesized and promoted as antitumor agents with therapeutic potential (10). These analogues are transported by the polyamine-transport system, and their therapeutic effects are less likely to be blocked by the availability of exogenous polyamines (11). The prostate has one of the highest polyamine concentrations compared with other tissues as a result of the high activities of polyamine-synthesizing enzymes present intracellularly in the gland (12). A direct correlation between ornithine decarboxylase activity (the key rate-limiting enzyme of polyamine biosynthesis) and tumor progression has been shown in the Dunning rat prostate tumor model (12).

Targeting polyamine analogues for cancer chemotherapy has taken a crucial turn because of the high cellular demand for polyamines and the active uptake (10, 13). Solid tumors, including prostate cancer, tend to be resistant to drug penetration by passive diffusion (14). The evidence that polyamine analogues seem to be actively concentrated in cells by a polyamine transporter (15, 16) provided a biochemical basis for the design of anticancer polyamine analogues that are similar enough in structure to the natural polyamines to be accepted by the transporter but have been structurally modified by the covalent linking of two bifunctional alkylating moieties to the backbone of the polyamine. The polyamine portion of this molecule provides the substrate...
for cellular polyamine transport, whereas the alkylating groups generate cytotoxicity presumably by covalently binding to DNA (17). The advantage of this type of design is that polyamine uptake systems are more active in cells with a high demand for polyamines, such as prostate cancer cells (12), thus providing high tumor selectivity. A second generation of compounds in this novel class of aziridine-containing polyamine analogues consist of the extension of the backbone to mimic spermine and the incorporation of two small alkylating moieties, aziridine functions, to provide DNA cross-linking ability and to generate cytotoxic activity (17, 18). The lead compound of this class of spermine analogues is rapidly taken up by the polyamine transporter and accumulates intracellularly by competitively inhibiting the uptake of spermine in cancer cells (18, 19).

The novel analogue BIS3 has demonstrated potent antitumor activity at submicromolar concentrations against leukemia and lung cancer cells (18). In this study, we investigated the tumor-targeted efficacy of BIS against androgen-independent human prostate cancer cells in vitro and in vivo. Our findings demonstrate a potent antitumor effect of this novel spermine analogue against prostatic tumors and its ability to increase the radiosensitivity of prostate cancer cells via the induction of apoptosis.

MATERIALS AND METHODS

Cell Lines. The human prostate cancer cell lines PC-3 (●) and DU-145 (○) were obtained from American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 (Life Technologies, Gaithersburg, MD), containing 10% FCS (Hyclone, Logan, UT) and 100 units/ml penicillin/streptomycin (Bio-Whittaker; Rockville, MD) in 5% CO2:95% air at 37°C.

Drugs. The spermine analogue BIS (NSC667005; patented) was synthesized and purified by Drs. P. Callery and Y. Li (Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy) and has recently been patented (20). BIS has a purity of more than 90% as determined by gas chromatography. The IC50 of BIS was determined from three different experiments using the Hill equation where Emax is the maximum effect (cytotoxicity), C is the concentration of BIS (µm), and H is the Hill Constant:

\[ E_{\text{max}} \times \frac{C^H}{IC_{50} + C^H} \]

and was defined as the concentration of the compound required to kill 50% of cells compared with that of untreated control cells. Cisplatin (Platinol) was obtained from Bristol Laboratories (Syracuse, NY).

Radiation Treatment. Exponentially growing cultures of PC-3 and DU-145 cells in either T-75 cm² culture flasks (starting density 10⁶ cells/flask) or 24-well plates were irradiated with a fixed dose rate of 172.2 cGy/min as determined by dosimetry. Analyses of cell viability in response to γ-irradiation was performed on total cell pools obtained from both attached and detached cells.

Clonogenic Survival Curves. Cells growing in six-well plates were exposed to single doses of ionizing irradiation (100, 300, 600, 900, and 1200 cGy) at 24-h postirradiation, and the colony forming ability was comparatively analyzed using total cell pools as described previously (21). Appropriate numbers of cells were plated in 60-mm dishes, and surviving colonies of 30 or more cells were counted after staining with crystal violet on day 7 of incubation.

Measurement of Apoptosis in Vitro. Prostate cancer cells undergoing apoptosis after treatment with BIS, irradiation or combination treatment were identified using the ApoTag Fluoroscein Kit (Oncor, Gaithersburg, MD), according to the manufacturer's instructions (21). Briefly, after exposure to anti-digoxigenin-fluorescein, cells were mounted on slides with propidium iodide. Cells were visualized under a fluorescence microscope (Axiovert-10; Zeiss, Thornwood, NY), using standard fluorescein excitation and emission filters. The percentage of apoptotic cells was derived from counting a total field of 200 cells (under high power); four different fields were counted from duplicate slides for each treatment.

In Vivo Efficacy Studies. C.B-17 male SCID mice, ages 6–8 weeks and virus-free, were purchased from Taconic (York, PA) and were allowed to acclimate to the animal facilities at the University of Maryland for 1 week. Mice were implanted i.c. with PC-3 tumor fragments from a mouse-passaged tumor (PC-3 mp²) on day 0. When the tumors reached a size of approximately 150 mm³, mice were assigned to treatment groups such that the mean and...
Tumor growth curves were established. Tumor regression was noted on the failure of tumor to grow on three successive measurements. Both body weights and caliper measurements were sent directly into Lotus 123 spreadsheets (Lotus 123, Lotus Development Corp., Cambridge, MA), through a RS 232 port interfaced using a gage port NT (Fred V. Fowler Co. Inc., Boston, MA) and Software Wedge (TAL Enterprises, Philadelphia, PA).

Measurement of Apoptosis in Vivo Tumors. On excision, tumors were fixed in 10% neutral buffered formalin. Tissues were subsequently embedded in paraffin, sectioned, and stained with H&E; serial sections were subjected to apoptosis analysis. The TUNEL assay was used to evaluate apoptosis in situ on formalin-fixed, paraffin-embedded tumor sections of s.c. tumors from mice treated after BIS treatment for 11 and 18 days (1.8 mg/kg) and from control animals as described previously (22). Counterstaining was performed using

median tumors for each group were equivalent. BIS was administered i.v. by lateral tail vein injections on twice weekly for 3 weeks. The doses of BIS used were 0.5, 0.8, 1.2, and 1.8 mg/kg, based on the pharmacokinetic data of tumor uptake. BIS at 1.8 mg/kg was the maximally tolerated dose for the multiple dosing studies. This dose was selected as a threshold on the basis of the observations that at higher doses (i.e., 2.5 mg/kg) on the same schedule, treated animals exhibited a significant loss of body weight and, upon necropsy, exhibited hepatotoxicity. At the above dosing regimen, there was no kidney toxicity observed. Cisplatin was administered i.p. as the positive control on the same schedule (2.5 mg/kg). Mice were observed twice daily, and tumor measurements, body weights, and the animal’s health status were recorded twice weekly (animals were dosed on exact body weight). Tumors were measured using a vernier caliper, and tumor volumes were calculated using the formula: $V = \frac{4}{3} \pi r^3$ where $L$ is the longest diameter and $W$ is the shortest diameter perpendicular to $L$. Mean and median days to tumor doubling were calculated as a measure of tumor growth rate. The time to one doubling was the time (in days) for a tumor to double its volume (e.g., from 300 to 600 mm$^3$). The efficacy of BIS treatment was compared with the cisplatin-treated group.

Fig. 3. A, the effect of ionizing irradiation on BIS cytotoxicity against human prostate cancer cells PC-3. Exponentially growing cultures of cells (24-well plates) that have been initially treated with increasing concentrations of BIS (0.01-30 μM) were exposed to ionizing irradiation (400 cGy). Cell viability was assessed at 3 days posttreatment. C, BIS alone ($IC_{50} = 0.72 \pm 0.12$ μM) BIS + ionizing irradiation ($IC_{50} = 0.02$). Values represent the mean of six points ± SE. B, the cytotoxic effect of γ-radiation alone on cell growth of human prostate cancer cells PC-3. Exponentially growing cultures of PC-3 cells were exposed to increasing doses of ionizing irradiation as indicated. Cell viability based on the trypan exclusion assay was determined at 3 days postirradiation. Values represent the mean of six points ± SE ($IC_{50} = 5.0$).

Fig. 4. The effect of BIS on clonogenic survival of irradiated PC-3 (A) and DU-145 (B) prostate cancer cells. Cells were treated with BIS (1 μM) for 2 h before exposure to increasing doses of ionizing irradiation as indicated. The clonogenic survival of BIS pretreated cells was comparatively analyzed at 24 h postirradiation with cells that were treated with radiation only.
RESULTS

The cytotoxic activity of BIS was quantified by determining the IC50 in the two androgen-independent human prostate cancer cell lines PC-3 and DU-145. PC-3 cells were more sensitive to the cytotoxic effects of BIS; the IC50 was 0.7 ± 0.12 μM, compared with the IC50 value obtained for DU-145 cells (1.2 ± 0.3 μM).

The dose response of BIS-mediated cytotoxicity against prostate cancer cells was established by the treatment of PC-3 and DU-145 cells with increasing concentrations of BIS (0.01–10 μM) for 3 days, and cell viability was determined using the trypan blue exclusion assay. The results shown in Fig. 1 demonstrate the effect of BIS on cell viability. The treatment of exponentially growing cultures of cells with BIS for 3 days resulted in a marked loss of cell viability in a dose-dependent pattern. Concentrations of BIS as low as 1 μM resulted in more than 90% cytotoxicity in both cell lines (Fig. 1).

The time course of BIS cytotoxicity against androgen-independent prostate cancer cells is shown in Fig. 2. After 3 days of treatment of PC-3 cells with BIS (1 μM), maximal cell killing (~90% cell loss) is observed. A similar pattern of dose-dependent loss of cell viability in response to BIS was observed for the DU-145 cells (data not shown). Because human prostate cancer cells undergo apoptotic cell death in response to ionizing irradiation (7, 21, 23), we subsequently determined the effect of BIS on the radiosensitivity of PC-3 and DU-145 prostate cancer cells. At 3 days posttreatment, as shown in Fig. 3A, the pretreatment of PC-3 cells with increasing concentrations of BIS before exposure to ionizing irradiation (400 cGy) led to a marked enhancement of BIS-induced cell killing as indicated by a significant decrease in the IC50 in the combination treatment to 0.02 (from a value of 0.7 for BIS alone). Fig. 3B represents the dose response of radiation-mediated cytotoxicity in PC-3 cells; 50% cytotoxicity was observed on treatment with 500 cGy at 3 days after irradiation. The IC50 (5.0 Gy), however, was considerably higher than the one observed for the combination treatment with BIS (Fig. 3A).

To investigate the effect of BIS treatment on the clonogenic survival of irradiated prostate cancer cells PC-3 and DU-145 cells were treated with BIS (1 μM) for 2 h before exposure to increasing pharmacologically relevant doses of ionizing irradiation. BIS treatment resulted in a dramatic induction of loss of clonogenic survival at lower radiation doses for both cell prostate cancer lines, PC-3 (Fig. 4A) and DU-145 (Fig. 4B).

The potential apoptotic nature of the cytotoxic effect of BIS on prostate cancer cells was investigated using the fluorescent terminal deoxynucleotidyl transferase assay in PC-3 cells after BIS treatment alone, radiation alone, or the combination of the two. As shown in Fig. 5, BIS treatment (1 μM) for 2 days resulted in a significant induction (~24%) of apoptosis of PC-3 cells. In response to ionizing irradiation alone (1,200 cGy), 15% of cells were identified as apoptotic at 2 days postirradiation. Pretreatment of PC-3 cells with BIS for 2 h before irradiation led to a significant increase (~40%) in the TUNEL-positive apoptotic cells (Fig. 5). These results suggest that BIS has an additive effect on radiation-induced apoptosis.

Treatment of tumor-bearing SCID mice with BIS had no significant effect on the body weight of the animals at any of the doses administered (data not shown). The slight decrease observed for the treated animals was a result of the decreased tumor weight caused by the antitumor effect of the drug. The results of the in vivo efficacy trials of BIS in human prostate cancer xenografts growing in SCID mice are shown in Fig. 6 for the PC-3 cells and in Fig. 7 for the DU-145 cells. Male SCID mice were implanted with PC-3 prostate tumor xenografts on day 0 and BIS was administered i.v. on day 10 at doses of 0.53, 0.8, 1.2, and 1.8 mg/kg (10 mice/treatment group). The tumor weight was examined at the termination of treatment (3 weeks), in untreated control, cisplatin treated (as a positive control), and BIS-treated animals. As shown in Fig. 6B, BIS treatment (at a dose of 1.8 mg/kg) resulted in a significant decrease in tumor weight of the PC-3 tumor xenografts compared with the cisplatin-treated or the nontreated control animals (P < 0.05). The 60% suppression of tumor volume/weight in the BIS-treated animals resulted in a dramatic six-fold increase in the tumor-doubling time from 1 to 6 days (data not shown). As shown in Fig. 7A, the rate of DU-145 tumor growth is markedly slower than that of the PC-3 cells (Fig. 6A). BIS treatment of the

Fig. 5. BIS enhances the apoptotic effect of radiation. Apoptotic cells were identified in situ 48 h after treatment with BIS alone (1 μM), radiation alone (1,200 cGy), or the combination of the two using the fluorescent TUNEL assay. Cells were visualized and quantified under epifluorescence illumination as described in "Materials and Methods." Nontreated control PC-3 cells are shown in the upper panel (insert, C). Data are expressed as the percentage of apoptotic cells identified in a population of 200–300 cells on each slide at ×400. Values represent the means obtained from two different slides for each treatment.
INDUCTION OF APOPTOSIS IN PROSTATE CANCER CELLS BY A POLYAMINE ANALOGUE

Minn 18 22 26 30 Days Post Tumor Implantation

Fig. 6. The in vivo effect of BIS treatment on tumor growth (A) and tumor weight (B) of PC-3 xenografts inoculated in SCID mice. Male SCID mice were implanted with PC-3 tumor fragments on day 0 and treatment with BIS was initiated on day 10 at indicated doses i.v. twice weekly. As a positive control, cisplatin was administered i.p. (2.5 mg/kg) on the same schedule. Negative controls included tumor-bearing animals that received i.v. injections of saline. Tumor volume was determined as described in “Materials and Methods.” BIS induced a significant suppression of tumor volume in a dose-dependent manner. The tumor weight was determined on termination of treatment and tumor excision. Values represent the mean ± SE from 10 different animals per treatment group (solid bars); median values are shown as shaded bars. ▲ Controls; ▼, Cisplatin; ○, Bis 1.8; □, Bis 1.2; △, Bis 0.8; ●, Bis 0.53; □, vehicle.

DU-145 prostate cancer xenografts (initiated on day 33 postinoculation) resulted in significant suppression of tumor growth (Fig. 7A) that was associated with a marked six-fold increase in the tumor-doubling time. At doses of 1.2 and 1.8 mg/kg, BIS had a significant antitumor effect compared with the vehicle control or with cisplatin treatment (P < 0.05; Fig. 7B).

We subsequently examined whether the treatment of tumor-bearing animals with BIS is characterized by the induction of apoptotic cell death in vivo. As revealed on Fig. 8, BIS treatment resulted in an increased number of TUNEL-positive cells in PC-3 prostate tumor xenografts. The number of apoptotic cells was significantly higher in tumors from animals that had been treated for 2 weeks with BIS (panel C) as compared with 1 week posttreatment (panel B) or with untreated control animals (panel A). Apoptotic bodies were also identified as basophilic-staining nuclei with condensed or fragmented appearance in tissue regions devoid of necrotic material. Quantitative analysis of this data is summarized in Table 1. As shown, there was a 10-fold induction in prostatic tumor cell apoptosis in vivo (as detected by the TUNEL assay) in the tumors of mice treated with BIS (1.8 mg/kg per dose), after 2 weeks of treatment compared with the untreated control (13% versus 1.2%, respectively; Table 1). Because the induction of apoptosis is an ongoing and rapid process in a tumor with individual tumor cells taking less than 60 min to complete the death process, a doubling in the number of apoptotic tumor cells may account for the observed tumor size reduction (Fig. 6B). These findings suggest that the induction of apoptosis increases prostate cancer cell killing and reduces tumor volume after treatment with novel spermine analogue BIS.

DISCUSSION

Targeting polyamine analogues for cancer chemotherapy emerges as a promising therapeutic strategy inasmuch as polyamines play an important role in the development and maintenance of neoplastic growth (9, 10, 24). A number of polyamine analogues with functional and structural similarities to spermine have been synthesized and investigated in a variety of solid tumors (25–27). Alkylating polyamines, by using the polyamine transporter for uptake into cancer cells, are likely to be highly effective against prostatic tumors by...
covalently binding to DNA. In the present study, we demonstrate a profound antitumor effect of a novel spermine analogue, BIS, against androgen-independent human prostate cancer cells in a dose-dependent pattern. The *in vivo* therapeutic efficacy of this novel drug BIS, tested in tumor-bearing mice, was remarkably effective with minimal host toxicity. Dramatic suppression of both PC-3 and DU-145 tumor xenografts was observed over a relatively short treatment period (3–4 weeks). Furthermore, our findings unequivocally indicate that the BIS prostate-targeted antitumor effect is mediated via induction of apoptosis both *in vitro* and *in vivo*. These observations are in full accord with recent studies suggesting that other polyamine analogues exert a potent apoptotic effect in several other human tumor cells including breast, non-small cell lung cancer cells, and melanoma cells (28–30).

Radiotherapy is considered a highly efficacious treatment for prostate cancer, with approximately 90% of prostatic tumors exhibiting a therapeutic response to radiotherapy (either external beam or brachytherapy). Understanding the key molecular pathways that modify the survival of tumor cells to radiotherapy will enable the development of specific pharmacological inhibitors of this response for the long-term management of prostate cancer patients. We and others have demonstrated previously (7, 21, 23) that both androgen-independent prostate cancer cell lines used in this study, PC-3 and DU-145, are able to undergo apoptosis in response to ionizing irradiation. The present results clearly indicate that BIS pretreatment significantly increases the radiosensitivity of prostate cancer cells by lowering the apoptotic threshold to ionizing irradiation. Our findings gain support from recent studies demonstrating that the activity of spermine analogues can significantly enhance the antitumor effect of radiation in human brain tumor cells (31).

The present study identifies the induction of apoptosis as a molecular mechanism underlying the antitumor effect of the novel spermine analogue BIS against human prostate cancer cells *in vitro* and *in vivo*. Recent evidence indicating that the apoptotic effect of a novel spermine analogue against human melanoma cells correlates with tumor regression *in vivo* (30) supports this concept. Unlike that study, however, in our study, the BIS analogue does not appear to have an effect on cell cycle progression (data not shown). Moreover, considering the recent reports demonstrating the cytostatic effect of other polyamine analogues against human prostate cancer cells (32, 33), one could argue that certain polyamine analogues that have a cytostatic effect on prostate cancer cells may exhibit different toxicity profiles and potencies from other analogues (such as our novel spermine analogue) that elicit cytoxic responses. The striking *in vivo* tumor-targeted apoptotic effect of BIS (with minimal toxicity) in human prostate cancer xenografts demonstrated in the present study makes our novel spermine analogue a promising candidate for clinical evaluation in advanced prostate cancer. Overall, our findings indicate an additive effect of a polyamine analogue and ionizing irradiation in activating the apoptotic process of prostate tumor cells. In view of the recent evidence implicating a pathway involving protein kinase C inhibition and sphingomyelinase activation in decreasing the apoptotic threshold of tumor cells (34), it is tempting to speculate on a similar molecular mechanism potentially underlying this BIS-mediated enhancement of radiation-induced cell killing. Clinical development of combination regimens of this novel spermine analogue with radiation therapy may provide a highly effective therapeutic approach that will potentially circumvent the need for intensified radiation delivery, minimize the toxicity of BIS, and ultimately result in an improved therapeutic index for patients with advanced prostate cancer. A similar combination therapeutic regimen has been proven successful in improving regional tumor control and patient survival in patients with head and neck tumors (35). This may be attributed to the effects of inhibition of polyamine biosynthesis on the volume of radiation-induced edema and necrosis compared with the untreated controls in patients with brain tumors (36).

Long-term studies are needed to determine whether the antitumor effects of BIS and ionizing irradiation will be additive *in vivo* with potential improvement of the therapeutic ratio. To this end, the combined effect of BIS at pharmacologically tolerated doses and ionizing irradiation is being investigated in human prostate cancer xenografts growing in SCID mice. Because *bcl-2* overexpression in prostate cancer cells is a reliable marker for a poor prognosis, resistance to radiotherapy, and tumor progression to hormone-refractory disease (37, 38), studies are presently in progress to examine whether overexpression of this potent

### Table 1: Induction of prostate cancer cell apoptosis in vivo following BIS treatment of PC-3 tumor xenografts-bearing mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Apoptotic indexa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1 wk)</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>BIS (1.2 mg/kg; 1 wk)</td>
<td>4.7 ± 1.2b</td>
</tr>
<tr>
<td>BIS (1.8 mg/kg; 1 wk)</td>
<td>5.5 ± 0.8b</td>
</tr>
<tr>
<td>Control (2 wk)</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>BIS (1.2 mg/kg; 2 wk)</td>
<td>8.9 ± 2.1b</td>
</tr>
<tr>
<td>BIS (1.8 mg/kg; 2 wk)</td>
<td>13.0 ± 0.7b</td>
</tr>
</tbody>
</table>

a Values represent mean ± SE.  
b Indicates a statistically SD from the values obtained for the control group.
apoptosis suppressor reverses the effect of BIS in enhancing the radio-
sensitivity of prostate cancer cells to apoptosis.

In summary, the data presented here demonstrate that pretreatment
with the novel spermine analogue BIS has the ability to decrease the
apoptotic threshold of prostate cancer cells to ionizing irradiation, thus
representing a novel strategy for overcoming radioresistance of pro-
static tumors. Direct molecular justification for such a concept stems
from a recent report indicating that increasing the sensitivity of tumor
cells to die via induction of apoptosis enhances the efficacy of
fractionated radiotherapy by reducing tumor clonogenic survival (39).
On the basis of the potent antitumor activity and minimized host
toxicity demonstrated by BIS, our goal is to continue preclinical
investigations to ultimately advance our spermine analogue toward a
clinical trial for prostate cancer patients with advanced disease. Other
polyamine analogues that are reported to be effective in other human
malignancies are undergoing clinical evaluation against solid tumors
(25) including prostate cancer (33).

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