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ORIGINAL ARTICLE

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Effects of the polyamine analogues BE-4-4-4-4, BE-3-7-3, and BE-3-3-3 on the proliferation of three prostate cancer cell lines

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Abstract *Purpose*: Polyamines are biologic cations necessary for normal cell growth. Polyamine analogues have been shown to be effective inhibitors of tumor growth. We tested the effect of the polyamine analogues 1,19-bis(ethylamino)-5,10,15-triazanonadecane (BE-4-4-4-4), N^1,N^{11}-bis(ethyl)norspermine (BE-3-3-3) and 1,15-bis(ethylamino)-4,12-diazapentadecane (BE-3-7-3) on the growth of the prostate cancer cell lines DU145, LNCaP and PC-3 in vitro. We also tested the effect of BE-4-4-4-4 on androgen-independent DU145 cells in vivo via a nude mouse xenograft model. *Methods*: In vitro, cell proliferation was measured using a DNA assay or a colony-formation assay. In vivo, mice were given saline or BE-4-4-4-4 3 mg/kg or 5 mg/kg intraperitoneally twice daily on days 7–10 and 14–17 (cycle 1), days 49–52 and 56–59 (cycle 2) and days 91–94 and 98–101 (cycle 3). *Results*: The proliferation of DU145, LNCaP and PC-3 prostate cancer cell lines was inhibited in a dose-dependent manner by BE-4-4-4-4. Intracellular putrescine, spermidine and spermine levels in all three cell lines declined after only 24 h exposure to BE-4-4-4-4 in vitro. Animals receiving BE-4-4-4-4 showed inhibition of tumor growth which continued throughout the experiment with 74% (3 mg/kg) and 81% (5 mg/kg) growth inhibition seen on day 101. No overt toxic reactions besides weight loss were observed in BE-4-4-4-4-treated animals. Tumor tissue from animals treated with BE-4-4-4-4 showed a dose-dependent decrease in spermidine and spermine levels but no decline in putrescine levels as compared with control. BE-4-4-4-4 levels were highest in tumors on day 63 with levels reaching 0.33 and 1.45 nmol/mg protein from animals treated at the 3 mg/kg and 5 mg/kg doses, respectively. *Conclusion*: These results show the polyamine analogues BE-4-4-4-4, BE-3-3-3 and BE-3-7-3 to be effective inhibitors of prostate cancer cell growth in vitro and BE-4-4-4-4 to be an effective inhibitor of DU145 cells in vivo with minimal toxicity.

Key words Polyamine · Prostate carcinoma · Nude mouse

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Introduction

The polyamines putrescine (PUT), spermidine (SPD) and spermine (SPM) are biologic cations present in all mammalian cells and are necessary for optimal development and growth [26, 27]. In the absence of supplemented polyamines, mutant mammalian and bacterial cell lines not able to produce polyamines show no growth or slowed growth [37]. Increased polyamine levels have been associated with rapid cell growth. Significant elevation of polyamines in carcinomatous tissue compared to adjoining normal tissue has been shown [8] and has stimulated the interest in polyamines in the search for antineoplastic agents.

Polyamines have been targeted at various points in their biosynthetic pathway and metabolism. Inhibitors of key polyamine biosynthetic enzymes such as

difluoromethylornithine (DFMO) [36], an inhibitor of ornithine decarboxylase that acts early in the polyamine biosynthetic pathway, and methylglyoxal bis(guanylhydrazone) (MGBG) [38], an inhibitor of *S*-adenosylmethionine decarboxylase, have shown antineoplastic activity in vitro with lesser effects seen in vivo [15, 26]. DFMO activity can be reversed in vitro with the addition of PUT to the medium [26]. Because of the ability of cells to take up extracellular polyamines, targeting polyamine biosynthesis is not as effective in vivo as in vitro. This has been further proven in studies in which a diet deficient in polyamines, when combined with antibiotics to rid the colon of polyamine-producing bacteria and a polyamine oxidase inhibitor to block the ability to recycle intracellular polyamines, increased the efficacy of polyamine depletion and tumor inhibition by DFMO [16, 22, 24, 32, 34].

A number of polyamine analogues have been designed to target polyamine functions. As cations, polyamines bond with a large number of cellular anions such as DNA and RNA, membrane phospholipids and cellular phosphoproteins [17]. This interaction can result in an alteration in the functional activity of the anion [22, 34, 37]. Polyamine analogues that enter cells and compete with natural polyamines at these binding sites without the ability to function as polyamines may act as antiproliferative agents [12]. Previous studies have shown the following polyamine analogues to be effective growth inhibitors in vitro and in vivo: 1,19-bis(ethylamino)-5,10,15-triazanonadecane (BE-4-4-4-4) against human brain [4, 22] and head and neck [14] tumor cells in vitro and against human glioma, lung and colon tumor xenografts in vivo [11]; 1,15-bis(ethylamino)-4,12-diazapentadecane (BE-3-7-3) against human brain tumor cells in vitro [3] and human leukemia and melanoma [5, 22, 31] cells in vitro and in vivo, and N^1,N^{11}-bis(ethyl)norspermine (BE-3-3-3) against human melanoma [22, 30] cells in vitro and in vivo. Also, phase I clinical trials are underway for BE-3-3-3 and BE-3-7-3 and are anticipated in the near future for BE-4-4-4-4 [22].

Prostate cancer incidence and mortality are on the rise. For the US, it is estimated that one in five men will develop invasive prostate cancer, and in 1996 alone, 41 000 men will die from prostate cancer [25]. With no effective treatment available for hormone-refractory prostate cancer, a novel treatment for this disease must be found. Since prostate tissue has one of the highest SPM concentrations of any tissue [21], we anticipated that BE-4-4-4-4, BE-3-7-3 and BE-3-3-3 would be effective inhibitors of growth in prostate cancer cell lines. We tested the effects of BE-4-4-4-4, BE-3-7-3 and BE-3-3-3 on cell proliferation in vitro using the prostate cancer cell lines DU145, PC-3 and LNCaP. The effect of BE-4-4-4-4 treatment on intracellular polyamine levels was measured in all three cell lines grown in culture. In vivo, the effects of BE-4-4-4-4 on the growth of androgen-independent DU145 cells [35] as tumor xenografts in the nude mouse model were tested.

Materials and methods

Materials

The established human prostate carcinoma cell lines DU145 [35], PC-3 [20] and LNCaP [18] were obtained from the American Type Culture Collection (Rockville, Md.). BE-4-4-4-4 and BE-3-3-3 were received from the National Cancer Institute (Bethesda, Md.). BE-3-7-3 was obtained from the Marion Merrell Dow Research Institute (Cincinnati, Ohio).

Cell and culture conditions

Cells were grown at 37 °C under humidified air containing 5% CO_2 in Dulbecco's Modified Eagle's Medium (DME) supplemented with 5% heat-inactivated fetal bovine serum (both GIBCO BRL products from Life technologies, Grand Island, N.Y.) and 1% antibiotic antimycotic solution (100×) (Sigma).

Animals

Male Hsd:athymic nude-*nu* (*nu*/*nu*, BALB/c origin) mice at 3–4 weeks of age were purchased from Harlan Sprague Dawley (Madison, Wis.) and allowed to acclimatize for 2 weeks prior to manipulation at 5–6 weeks of age. Mice were handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH number 85–23, 1985). The mice were housed under laminar air flow in an isolated animal unit in rooms maintained at 25–28 °C and 60–70% humidity with a 12-hour light/dark cycle. A maximum of four mice were housed per sterilized cage with free access to sterilized water and food.

The mice received subcutaneous injections of 2.5×10^5 DU145 cells in DME at two sites in the ventral fat pads. Body weight and tumor size were measured twice weekly. Two perpendicular diameters of each tumor were measured with a caliper and tumor volume was calculated using the method of the National Cancer Institute [13]: length × width$^2/2$ = volume (mm^3). All groups were compared using a heteroscedastic two-sided Student's *t*-test.

Mice were given three cycles of 0.9% saline (control group) or BE-4-4-4-4 in 0.9% saline, pH 7.4 (adjusted with 0.1 *M* $NaHCO_3$), sterile filtered as daily treatment aliquots 24 h prior to the start of each treatment cycle and frozen at −20 °C until the day of use. Doses of 3 mg/kg or 5 mg/kg body weight were injected intraperitoneally (i.p.) twice daily 8 h apart on days 7–10 and 14–17 (cycle 1), days 49–52 and 56–59 (cycle 2), and days 91–94 and 98–101 (cycle 3) with day 0 being the day of cell injection. Animals bearing tumors were euthanized when the tumor burden reached 10–15% of body weight. Animals obviously ill/cachectic or with ulcerated tumors prior to reaching this level of tumor burden were euthanized immediately. Animals to be euthanized received etomidate (Amidate, Abbot Laboratories, North Chicago, Ill.) 25 to 30 mg/kg i.p., were bled via orbital stick for plasma collection, then euthanized via cervical dislocation. Animals were examined for any overt toxicity. Tumors were harvested and stored frozen for polyamine level analysis.

Cell proliferation assay

The effects of BE-4-4-4-4, BE-3-7-3 and BE-3-3-3 on cell proliferation were measured via DNA quantitation [33] and an anchorage-dependent clonogenic assay [39]. On day −1, cells were harvested by trypsinization (trypsin-EDTA; GIBCO, Grand Island, N.Y.), resuspended in complete medium and counted on a standard hemacytometer. For the DNA assay, cells were plated 1000 cells/0.1 ml per well in 96-well microtiter plates (Corning Glass Works, Corning, N.Y.). The anchorage-dependent colony-formation assay was performed in six-well plates (Becton Dickinson, Lincoln Park, N.J.) with 500 cells/1.5 ml per well. On day 0, equal volumes of medium

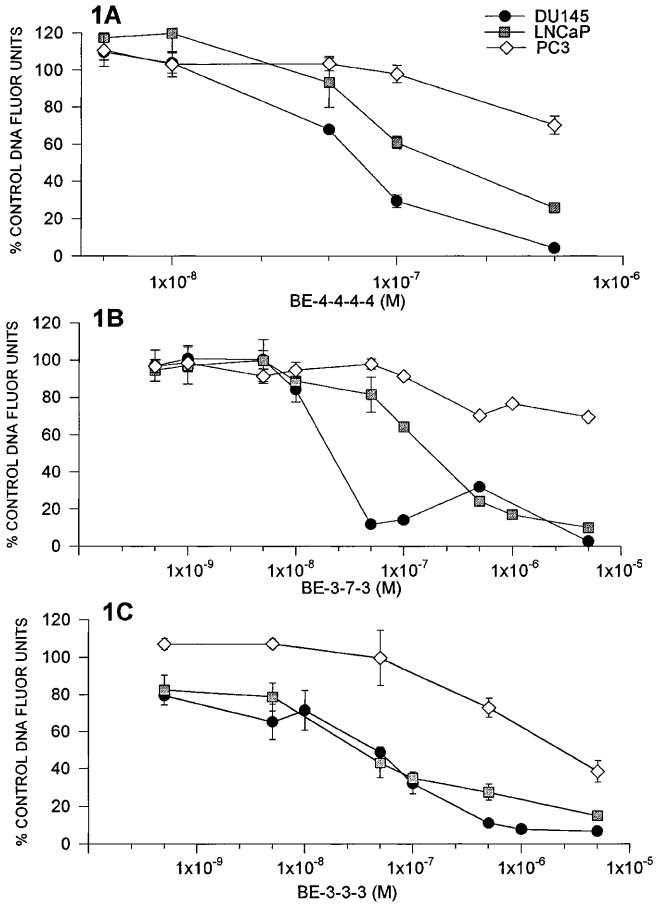

Fig. 1A–C Effects of varying concentrations of BE-4-4-4-4 (**A**), BE-3-7-3 (**B**) or BE-3-3-3 (**C**) on the growth of DU145 (●), LNCaP (□) and PC-3 (◇) prostate cancer cell lines in vitro. Cells were harvested on day 6 of treatment with analogue and proliferation was measured using a DNA assay. The values are the means ± SD of eight replicate wells expressed as a percentage of control (no analogue added)

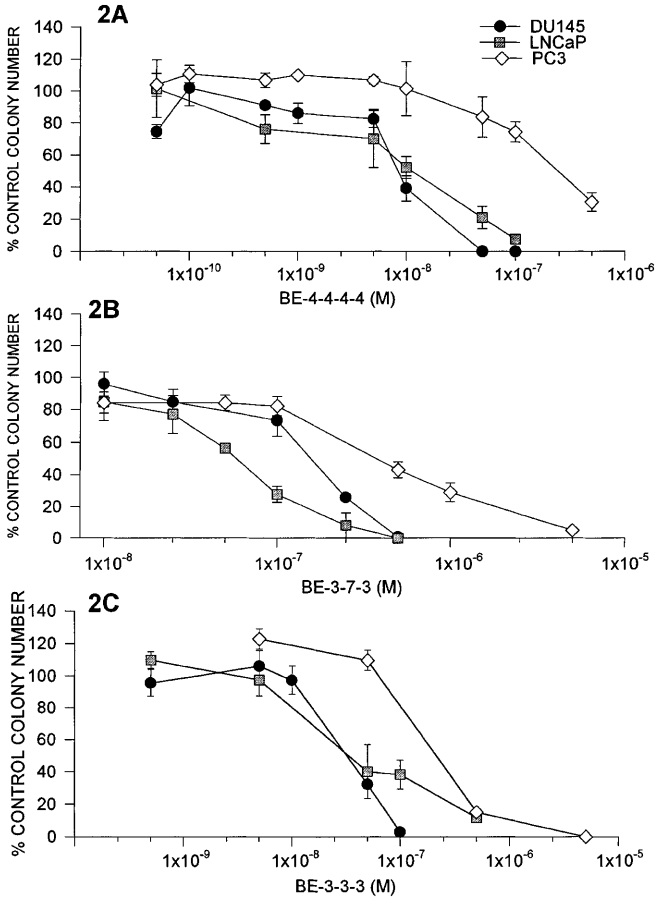

Fig. 2A–C Effects of varying concentrations of BE-4-4-4-4 (**A**), BE-3-7-3 (**B**) or BE-3-3-3 (**C**) on the growth of DU145 (●), LNCaP (□) and PC-3 (◇) prostate cancer cell lines in vitro. Cells were harvested on day 8 of treatment with analogue and proliferation was measured using a colony formation assay. The values are the means ± SD of three replicate wells expressed as a percentage of control (no analogue added)

containing a 2× concentration of the appropriate BE-4-4-4-4, BE-3-7-3 or BE-3-3-3 treatments (taken from 10 mg analogue/ml saline frozen stock solution) were added to each well for a total volume of 0.2 ml (DNA) or 3.0 ml (colony formation).

The DNA assay [33] was performed with the following modifications. Plates were harvested on days 0, 1, 3 and 6. Medium was poured from the plates. Wells were washed once with 0.2 ml 25% phosphate-buffered saline (PBS), 0.1 ml distilled water was added to each well and plates were incubated for at least 1 h at room temperature. Plates were then stored at −80 °C until final analysis. Frozen plates were thawed and upon reaching room temperature 0.2 ml Hoescht 33258 fluorochrome dye at 10 μg/ml in a Tris/NaCl/EDTA solution (TNE) was added to each well (final fluorochrome concentration 6.67 μg/ml). Fluorescence was measured on an automated 96-well plate reader (CytoFluor 2350; Millipore Intertech, Bedford, Mass.) and expressed in DNA fluorescence units.

Anchorage-dependent colony formation [39] was measured on day 8 by staining the plates with crystal violet. Colonies consisting of > 50 cells were counted.

Polyamine analysis

The effects of BE-4-4-4-4 on intracellular polyamine levels of prostate cancer cells in vitro and in vivo were measured [19]. Briefly, in vitro, DU145, LNCaP or PC-3 cells were plated at 2.2×10^5 cells/100 mm plate. After a 24-h incubation, plates were treated with various doses of BE-4-4-4-4. At 0, 24, 72 and 144 h, cells were harvested by trypsinization, washed twice with cold PBS, and counted on a standard hemacytometer. Cells were sonicated in 0.3 to 2.0 ml 2% perchloric acid and centrifuged. In vivo, approximately 50 mg xenograft tumor tissue described above was weighed out and placed in a chilled glass tube containing 1 ml 2% perchloric acid. Tissues were homogenized with a polytron, sonicated, allowed to sit on ice for 1 h and centrifuged. The supernatant was separated from the pellet and samples were stored at −80 °C until analysis. Upon analysis, supernatant was thawed and 0.2 ml of sample was dansylated and analyzed by reverse-phase high-pressure liquid chromatography (HPLC) as previously described [19]. The protein content of sample pellets was measured using the assay of Bradford [6]. Polyamine levels are expressed as nanomoles per milligram protein. All groups were compared using a heteroscedastic two-sided Student's *t*-test.

Results

In vitro growth inhibition

Drug concentrations which inhibited growth 50% of control (IC_{50}) were rounded to the nearest half log. Comparison of IC_{50} values between cell lines showed

BE-4-4-4-4, BE-3-7-3 and BE-3-3-3 to be equally effective in inhibiting the growth of DU145 and LNCaP cells in both the DNA (Fig. 1) and colony-formation (Fig. 2) assays. IC_{50} values for each analogue were within one-half log of each other, ranging from 5×10^{-8} to $1 \times 10^{-7} M$ in the DNA assay and from 1×10^{-8} to $1 \times 10^{-7} M$ in the colony-formation assay for DU145 and LNCaP cells, respectively. PC-3 cells required at least one and up to two logs more of each analogue with IC_{50} values ranging from 1×10^{-6} to $1 \times 10^{-5} M$ in the DNA assay and from 5×10^{-7} to $1 \times 10^{-6} M$ in the colony-formation assay. Within the same cell line, IC_{50} values for DU145 cells and PC-3 cells treated with BE-3-7-3 were one log higher than for those treated with BE-4-4-4-4 in the colony-formation assay and DNA assay, respectively. Inhibition of growth to less than 10% of control occurred in DU145 cells treated with $5 \times 10^{-7} M$ BE-4-4-4-4, $5 \times 10^{-6} M$ BE-3-7-3 or $1 \times 10^{-6} M$ BE-3-3-3 measured by the DNA assay. The colony-formation assay showed DU145 cells to be inhibited to less than 10% of control at $5 \times 10^{-8} M$ BE-4-4-4-4, $5 \times 10^{-7} M$ BE-3-7-3 or $1 \times 10^{-7} M$ BE-3-3-3. At these concentrations, neither PC-3 nor LNCaP cells reached this level of inhibition.

In vitro polyamine levels

Each value is the average of two samples from separate experiments. Uptake of BE-4-4-4-4 into all three cell lines was dose-dependent. After treatment with $5 \times 10^{-7} M$ BE-4-4-4-4, levels of BE-4-4-4-4 reached 5.10, 1.33, and 4.57 nmol/mg protein in DU145, PC-3 and LNCaP cells, respectively, at 24 h (Table 1) and remained high throughout the experiment with levels of 4.15, 2.41, and 5.82 nmol/mg protein, respectively, at 144 h (data not shown). At the $1 \times 10^{-7} M$ and $5 \times 10^{-8} M$ treatment levels, BE-4-4-4-4 levels peaked at 24 h for all cell lines, but were not maintained at 72 and 144 h. BE-4-4-4-4 levels in the cells treated at the $5 \times 10^{-11} M$ dose level were no different from the untreated cells for all cell lines at all time-points.

Polyamine depletion generally occurred in a manner which was dependent upon the concentration of BE-4-4-4-4 in the medium and upon the amount of BE-4-4-4-4 taken into the cell. Polyamine levels in cells treated with $5 \times 10^{-7} M$ BE-4-4-4-4 showed decreased levels of PUT, SPD and SPM by 24 h with levels reduced to 31%, 27% and 31% of control, respectively, for LNCaP cells; to 18%, 29% and 61% of control, respectively, for PC-3 cells; and to nondetectable levels of PUT and to 13%

Table 1 Intracellular polyamine levels in cells treated with BE-4-4-4-4 in vitro. Values are an average of two points from separate experiments (*ND* not detectable; *PUT* putrescine; *SPD* spermidine; *SPM* spermine)

Cell line	Time-point (hour)	BE-4-4-4-4 treatment × (*M*)	Polyamine level (nmol/mg protein)			
			PUT	SPD	SPM	BE-4-4-4-4
DU145	0	0	0.45	3.78	4.20	ND
		5×10^{-11}	0.52	4.64	4.75	ND
		5×10^{-8}	0.35	4.48	4.67	0.04
		1×10^{-7}	0.38	4.54	4.84	0.02
		5×10^{-7}	0.50	2.00	5.08	0.16
	24	0	0.14	3.95	6.78	0.01
		5×10^{-11}	0.13	4.07	6.95	ND
		5×10^{-8}	0.08	2.85*	5.76	1.08*
		1×10^{-7}	0.03	2.04*	4.93*	2.20*
		5×10^{-7}	ND	0.52*	2.31*	5.10*
PC-3	0	0	1.30	2.47	5.44	0.02
		5×10^{-11}	1.58	2.88	6.97	ND
		5×10^{-8}	1.19	2.63	6.33	0.02
		1×10^{-7}	1.07	2.29	5.89	0.01
		5×10^{-7}	1.40	2.74	6.86	ND
	24	0	0.62	1.77	6.86	ND
		5×10^{-11}	0.72	1.83	6.83	0.01
		5×10^{-8}	0.60	1.49	6.61	0.38
		1×10^{-7}	0.41	1.18	6.23*	0.62*
		5×10^{-7}	0.11*	0.52	4.19	1.33
LNCaP	0	0	0.46	7.93	14.22	0.22
		5×10^{-11}	0.40	6.25	21.56	0.19
		5×10^{-8}	0.34	6.05	20.14	0.35
		1×10^{-7}	0.44	4.33	14.60	0.23
		5×10^{-7}	0.36	5.08	19.46	0.36
	24	0	0.16	2.48	11.90	0.03
		5×10^{-11}	0.32	3.59	15.09	0.13
		5×10^{-8}	0.14	2.16	10.78	1.06
		1×10^{-7}	0.13	1.81	10.82	1.95
		5×10^{-7}	0.05	0.66	3.73	4.57*

*$P < 0.10$ vs untreated cells at same time-point

(SPD) and 30% (SPM) of control for DU145 cells. At 24 h, cells treated with $5 \times 10^{-8} M$ or $1 \times 10^{-7} M$ BE-4-4-4-4 showed a decrease in polyamine levels, but did not reach the level of depletion observed in cells treated with $5 \times 10^{-7} M$ BE-4-4-4-4. For all three cell lines, cells treated with $5 \times 10^{-11} M$ BE-4-4-4-4 show no significant difference in polyamine levels as compared with control. Overall, polyamine depletion appeared to be dose-dependent throughout the experiment, with DU145 cells being most sensitive to BE-4-4-4-4 treatment.

Tumor growth inhibition

Tumor volumes (TV) from the various treatment groups were compared with the control group TV as percent inhibition calculated from the following equation: % inhibition = [(average TV treated/average TV control) $-1] \times 100$. On day 17, the last day of the first cycle of treatment, animals receiving BE-4-4-4-4 showed 46% (3 mg/kg body weight) and 63% (5 mg/kg) tumor growth inhibition (Fig. 3A). On day 31, maximum inhibition of 74% (3 mg/kg) and 91% (5 mg/kg) was achieved. Tumor growth inhibition was maintained throughout the experiment with 74% (3 mg/kg) and 81% (5 mg/kg) seen on day 101, the last day of treatment.

Animals treated with 5 mg/kg BE-4-4-4-4 showed a 6% weight loss on day 21, with full recovery before the second cycle of treatment (Fig. 3B). Following the second cycle, this group showed a 17% weight loss on day 63 with recovery reaching 91% before the third cycle of treatment. After the third cycle of treatment, a maximal weight loss of 21% was seen on day 108. Animals treated with 3 mg/kg BE-4-4-4-4 showed weight loss after the second cycle of treatment reaching 8% on day 63 with full recovery seen before the third cycle and no significant weight loss seen after the third cycle of treatment. No significant weight loss was seen in the control group or in animals given no treatment throughout the experiment.

Tumor tissue polyamine levels

BE-4-4-4-4 uptake was evident in all tumor tissues from BE-4-4-4-4-treated animals. Of all time-points where tumor tissue was available for polyamine level analysis, the highest levels of BE-4-4-4-4 were measured on day 63, 4 days after the last treatment day in the second cycle, with levels reaching 0.33 and 1.45 nmol/mg protein in tumors from animals treated at 3 mg/kg and 5 mg/kg, respectively (Table 2). Levels of BE-4-4-4-4 in tumor tissue appeared to be dose-dependent throughout the experiment with the highest levels occurring in the group treated at 5 mg/kg. On day 63, tumors from the group treated at 5 mg/kg showed decreased levels of SPD at 1.95 nmol/mg protein versus 2.33 nmol/mg protein in control tumors and decreased levels of SPM at 0.65 nmol/mg protein versus 1.59 nmol/mg protein in control tumors. A decrease in SPD and SPM levels in tumors from the group treated at 5 mg/kg as compared with the control group appeared at all time-points where tumor polyamine levels were measured throughout the experiment. Generally, levels of SPD and SPM in the group treated at 3 mg/kg were also less than the levels in the control group, but were not reduced as much as in the group treated at 5 mg/kg. Overall, PUT levels in tumors from treated groups were not depleted.

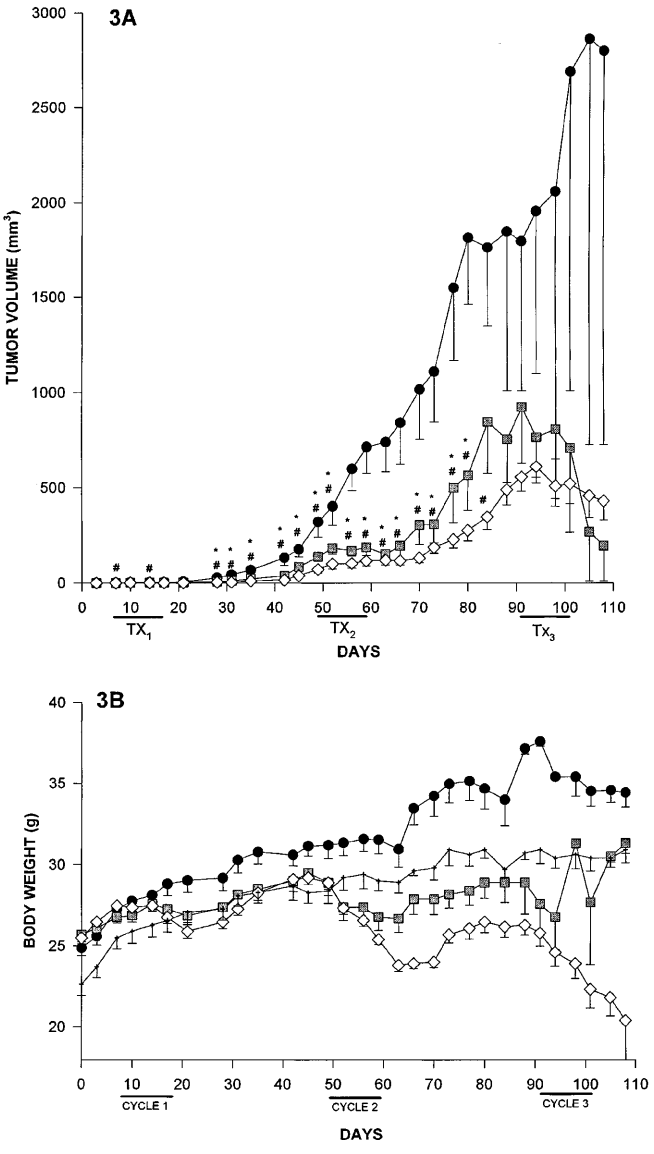

Fig. 3A,B Tumor growth rate of DU145 xenografts (**A**) and body weights of mice (**B**) treated with BE-4-4-4-4. Nude mice carrying DU145 xenograft tumors were given twice daily i.p. injections of saline (●) or BE-4-4-4-4 3 mg/kg (□) or 5 mg/kg (◇) on days 7–10 and 14–17 (cycle 1), days 49–52 and 56–59 (cycle 2), and days 91–94 and 98–101 (cycle 3). Control animals (+) received no cell injections or treatments throughout the experiment ($n = 3$). Tumor growth is expressed as tumor volumes (mm^3). The values are the mean tumor volume ($n = 2$ to 24) or body weight ($n = 2$ to 12) (*bars* SE). $^{*}P \leq 0.05$ for tumors from animals treated at 3 mg/kg vs tumors from saline-treated animals; $^{\#}P \leq 0.05$ for tumors from animals treated at 5 mg/kg vs tumors from saline-treated animals

Table 2 Polyamine levels in tumors from mice treated with BE-4-4-4-4. Values where $n > 1$ are the average polyamine levels in two or more separate tumors (*ND* not detectable; *PUT* putrescine; *SPD* spermidine; *SPM* spermine)

Treatment	Day	*n*	Polyamine level (nmol/mg protein)			
			PUT	SPD	SPM	BE-4-4-4-4
Saline	63	5	0.64	2.33	1.59	ND
3 mg/kg	63	2	0.72	3.40	1.45	0.33
5 mg/kg	63	2	0.77	1.95	0.65	1.45
Saline	70	2	0.58	2.66	1.50	ND
3 mg/kg	73	1	0.50	1.84	1.23	0.13
5 mg/kg	73	1	0.42	0.70	0.26	0.32
Saline	84	8	0.56	2.26	1.58	ND
3 mg/kg	97	5	0.72	1.96	1.30	0.27
5 mg/kg	97	3	0.70	1.61	0.90	0.61
Saline	*	*	*	*	*	*
3 mg/kg	101	2	0.29	1.29	1.06	0.30
5 mg/kg	101	5	0.41	1.34	0.88	0.92

Discussion

Polyamine analogues have been shown to be effective inhibitors of many different cell lines both in vitro and in vivo [3, 4, 5, 11, 14, 22, 30]. Our results show the polyamine analogues BE-4-4-4-4, BE-3-7-3 and BE-3-3-3 to be effective growth inhibitors of the prostate cancer cell lines DU145, LNCaP and PC-3 in vitro. The mechanism by which polyamine analogues inhibit growth is still unclear and widely studied. One proposed mechanism is polyamine depletion [26]. Other proposed mechanisms of the action of polyamine analogues are alterations in the activity of the polyamine catabolizing enzyme SSAT [7, 26, 27, 29], induction of programmed cell death [23] and modification of DNA conformation [1, 2, 10], which may be preliminary effects that in turn cause intracellular polyamine depletion. BE-4-4-4-4 was effective in decreasing the intracellular polyamine levels in all three prostate cancer cell lines, with DU145 cells showing the most sensitivity to growth inhibition and having the greatest overall polyamine depletion with BE-4-4-4-4 treatment. PC-3 cells treated with BE-4-4-4-4 showed lower intracellular analogue levels with less polyamine depletion and less growth inhibition than treated LNCaP and DU145 cells, which suggests that the amount of growth inhibition is dependent on the analogue levels in the cell. At 0 h, the various treatments of BE-4-4-4-4 were put onto the plates then immediately removed. The small amount of BE-4-4-4-4 seen in all three cell lines at 0 h reflects the background measurement inherent to the assay.

The androgen-independent prostate cancer cell line DU145 has been previously established in a nude mouse xenograft model in our laboratory. The growth of this cell line also appeared to be more sensitive to polyamine analogue treatment than that of the androgen-independent PC-3 cell line. Of the three analogues tested in vitro, BE-4-4-4-4 has been the most extensively studied for its mechanism of action and its effects on the growth of various cell lines in vitro and in vivo [4, 11, 14, 22]. Overall, BE-4-4-4-4 was most effective in inhibiting in vitro growth of the prostate cancer cell lines tested in our laboratory. On this basis, we tested the effect of BE-4-4-4-4 on DU145 cells grown in vivo. BE-4-4-4-4 effectively inhibited the growth of DU145 xenograft tumors. Animals receiving 5 mg/kg BE-4-4-4-4 showed significant weight loss with no full recovery after the second and third cycles of treatment. Animals appeared to tolerate 3 mg/kg BE-4-4-4-4 with only a slight weight loss occurring after the second cycle with full recovery before the third cycle of treatment. Average body weight of the group treated at 3 mg/kg did not significantly vary from the average body weight of the untreated group. Although tumor growth in the group treated at 5 mg/kg was less than the tumor growth in the group treated at 3 mg/kg throughout the experiment, the 3 mg/kg BE-4-4-4-4 treatment appeared to be optimal, with minimal weight loss and effective growth inhibition throughout the experiment.

BE-4-4-4-4 was also shown to be effective in decreasing the tumor polyamine levels. This effect correlated with the in vitro data with the exception of the effect on PUT levels. Although the levels of PUT were depleted in vitro, in vivo the levels remained unchanged between BE-4-4-4-4-treated and untreated groups. One explanation may be the uptake of exogenous PUT derived from the diet and the normal flora of the intestinal tract [24]. Although all polyamines are available via this route, SPD and SPM remained depleted in BE-4-4-4-4-treated tumors. This suggests that BE-4-4-4-4 may affect cell uptake of SPD and SPM, that these compounds are retained in the cell, or that BE-4-4-4-4 interferes with polyamine biosynthesis. BE-4-4-4-4 was detected in tumors from animals treated with both 3 mg/kg and 5 mg/kg BE-4-4-4-4 up to 14 days after the last day of the second cycle of treatment, indicating that BE-4-4-4-4 is retained in the tumor cells for a significant period of time.

Adenocarcinoma of the prostate is one of the leading causes of death in males. This high rate of mortality is associated with widespread metastatic disease within the first year of diagnosis in stage D patients [9]. Polyamine

analogues represent a new approach to prostate cancer therapy. Polyamine analogues such as BE-4-4-4-4, BE-3-7-3 and BE-3-3-3 have the ability to slow prostate carcinoma growth, which may prevent progression of the disease to the metastatic stage, or may prevent metastases from proliferating, with minimal toxicity. Polyamine analogues may also provide beneficial effects in combination with current commonly used therapies. Preclinical studies have shown the polyamine biosynthesis inhibitor DFMO in combination with radiation therapy to be promising in head and neck squamous cell carcinoma cells [28]. Combinations of polyamine analogues with other antineoplastic polyamine biosynthesis inhibitors such as DFMO and MGBG may enhance the effect of those compounds. These analogues may allow the patient to survive the disease without a cure. BE-3-3-3 and BE-3-7-3 are currently being tested in the clinic and it is hoped that BE-4-4-4-4 will soon follow [22]. Although all three analogues have been shown to be effective in inhibiting the growth of prostate cancer in vitro, BE-4-4-4-4 has been most extensively studied in our laboratory. Further research into combining BE-4-4-4-4 with other anticancer therapies is warranted.

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