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Antitumor effects of *N*-alkylated polyamine analogues in human pancreatic adenocarcinoma models*

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Summary. Adenocarcinoma of the pancreas presents a formidable challenge both experimentally and clinically, whereby effective anticancer therapy is lacking. We have recently explored a relatively new class of antitumor agents in pancreatic cancer cell lines and have found the bis-ethyl derivatives of spermine to show considerable promise. In the present paper, we report the results of in vivo studies demonstrating the antitumor activity of two of these *N*-alkylated analogues, N^1,N^{14}-bis(ethyl)homospermine (BEHSPM) and N^1,N^{11}-bis(ethyl)norspermine (BENSPM) in athymic (nude) mouse xenografts of two human pancreatic ductal adenocarcinoma cell lines, PANC-1 (poorly differentiated) and BxPC-3 (moderately well-differentiated). BENSPM was found to exert greater antitumor activity in vivo than either BEHSPM or other conventional agents, largely because higher doses could be given due to its lower toxicity to mice. BENSPM shows greater activity than any other agent we have thus far tested against our pancreatic-cancer models. Optimal schedules of administration have yet to be determined. Nevertheless, of the analogues tested, BENSPM presently appears to be the analogue of choice for further development.

Introduction

Pancreatic adenocarcinoma is notorious for its relative resistance to available therapeutic modalities. Despite claims that nihilism should be avoided in evaluating the disease [15], conventional therapy offers at best a few months' prolongation of life and some palliation of symptoms [13, 16, 17]. We have explored polyamine inhibitors as promising experimental approaches to the treatment of pancreatic cancer [7–9]. Recently, analogues of naturally occurring polyamines have been developed and found to exert encouraging antiproliferative activity when tested against pancreatic-adenocarcinoma cell lines in culture [11, 12]. These bis-ethyl derivatives of spermine have also been shown to be active against human brain tumor [1], certain lung cancer [6], colon carcinoma [20], and melanoma cell lines [22]. In pancreatic cancer cell lines and certain other cells, N^1,N^{11}-bis(ethyl)norspermine (BENSPM) is more potent than N^1,N^{14}-bis(ethyl)homospermine (BEHSPM) in suppressing the major biosynthetic enzymes ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) and in inducing the interconversion enzyme spermidine/spermine N^1-acetyltransferase (SSAT) [12]. However, the correlation of SSAT induction with antiproliferative activity varies among the tumor models tested [6, 12, 22; Bergeron et al., submitted for publication].

The current study documents the in vivo activity of BENSPM and BEHSPM in established (250–350 mm^3) athymic (nude) mouse xenografts of two human pancreatic ductal adenocarcinoma cell lines, PANC-1 (poorly differentiated) and BxPC-3 (moderately well-differentiated). In BxPC-3, BEHSPM and BENSPM were compared with two regimens commonly used in the treatment of clinical pancreatic adenocarcinoma – 5-fluorouracil (5FU) alone and the combination of 5FU, doxorubicin (Adriamycin), and mitomycin C (FAM). At the highest dose used, BENSPM was found to display greater antitumor activity than the other analogues or regimens tested. BENSPM was extremely well tolerated and is the most active agent we have tested to date in our models of pancreatic cancer.

Materials and methods

Materials. BENSPM and BEHSPM were synthesized as hydrochloride salts as previously described [2]. 5FU and doxorubicin were obtained commercially. Mitomycin C was kindly supplied by Bristol-Myers.

Tumor models. The characteristics of the cell lines used in this study have been described elsewhere [11, 18, 19]. PANC-1 and BxPC-3 were obtained from the American Type Culture Collection (Rockville, Md.).

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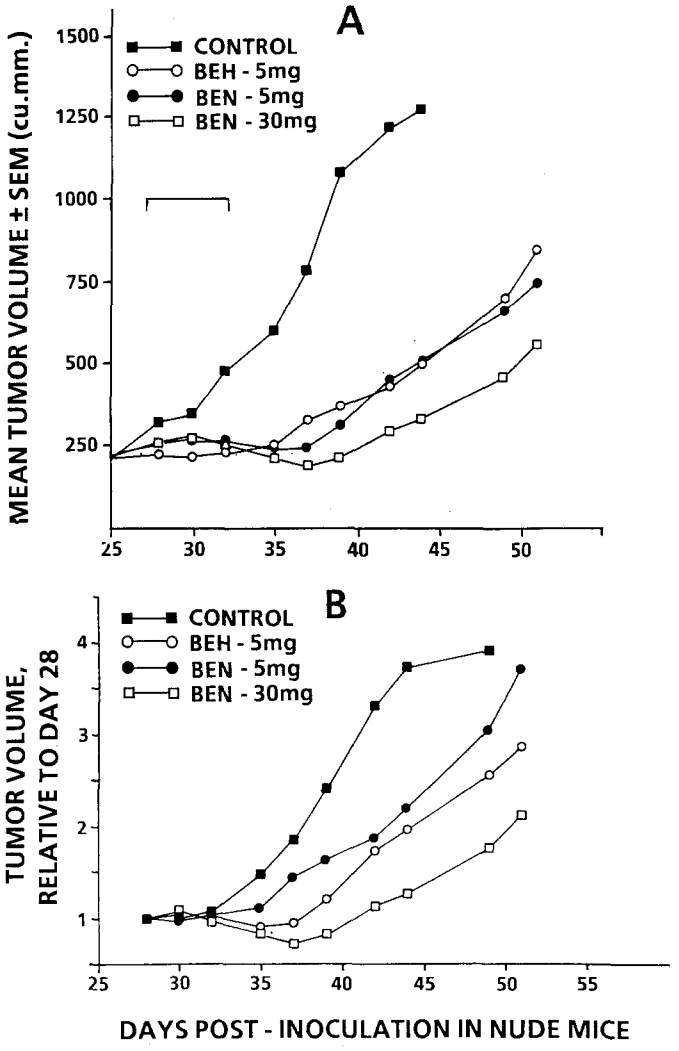

Fig. 1 A, B. Comparison of the in vivo antitumor effects of BEHSPM and BENSPM (at 2 dose levels) against athymic (nude) mouse xenografts of PANC-1. (See Table 1 for details of the therapy.) Treatment extended from day 27 through day 32 as indicated by the *bar* in **A**. **A** actual tumor volumes and growth curves. **B** Tumor volumes relative to those measured on day 28. Each group comprised 10 animals

Athymic (nude) mouse xenografts were developed from the cell lines by the s.c. implantation of 10^7 cells into the shoulder region of 4- to 5-week-old male nude mice. After approximately 4 months, palpable tumors developed, and these tumors were subsequently passaged by trocar implantation of 1 mm fragments of the solid tumors. The tumors were passaged once in nude mice prior to their use in the in vivo experiments. Palpable, measurable tumors were obtained within 2–3 weeks and treatable tumors, in 3–4 weeks, with BxPC-3 growing somewhat slower than PANC-1. The tumor growth rate on the second (experimental) passage was similar to that on the first passage of the tumors from solid tumor fragments (as opposed to direct cell inoculation). Histology was verified from each tumor passage. Tumors were measured in three dimensions without correction for skin thickness, and tumor volume was calculated according to the formula for a hemiellipsoid [23]:

$$\text{Volume} = {}^1/_2\,(4\pi/3)(l/2)(w/2)(h) = 0.5236lwh,$$

where l represents length; w, width; and h, height. Animals were weighed on the same days on which tumors were measured three times weekly (Monday, Wednesday, and Friday). Treatment was begun when average tumor volumes had reached 250–350 mm^3.

Results

The response of PANC-1 xenografts to BEHSPM and to two doses of BENSPM is shown in Fig. 1, and that of BxPC-3 to BEHSPM, BENSPM, 5FU, and FAM is shown in Fig. 2. Several measures of antitumor response are summarized in Table 1. All analogues were given i.p. q 8 h for 6 days. The dose of BEHSPM was considered to be maximal, since animals treated at doses higher than 5–6 mg/kg q 8 h suffered significant weight loss [5]. The 30 mg/kg q 8 h dose of BENSPM used in the present study was considered to be maximal at the time the experiments were conducted, and the 5 mg/kg q 8 h dose was included for comparison with BEHSPM. However, no toxicity was found at the highest dose of BENSPM as shown in Fig. 3, and it was subsequently found that higher doses of BENSPM could be tolerated by nude mice [5]. Nevertheless, the activity of BENSPM was impressive in the two pancreatic-tumor models tested.

The assessment of responses to the polyamine analogues and the other agents tested are shown in Table 1. Actual tumor regressions amounting to 33% and 36% of the pretreament volume were induced in PANC-1 and BxPC-3 by BENSPM given at 30 mg/kg q 8 h. Estimates of the log cell kill obtained using the method of Corbett et al. [14] are also shown in Table 1. In BxPC-3 xenografts, these estimates of log cell kill show little difference between the polyamine analogues and the conventional chemotherapeutic regimens and are more conservative in nature than are the findings of the other assessment of antitumor activity noted in Table 1, i.e., tumor regression. However, tumor regression is the criterion used in clinical trials to assess efficacy and is, in our opinion, the more relevant and dramatic measure of antitumor response to BENSPM. Although the regression documented for BENSPM in vivo was transitory, it should be pointed out that this analogue could have been given for longer periods and/or at higher doses and that the actual antitumor activity we noted is thus lower than that which would be expected at an optimal dose and/or schedule.

Discussion

bis-Ethyl analogues of spermine have been shown to inhibit cell proliferation and depress polyamine pools in human brain tumor [1], lung-cancer [6], colon carcinoma [20], melanoma [22], and pancreatic adenocarcinoma cell lines [12] as well as the rodent murine L1210 leukemia [3], Chinese hamster ovary (CHO) [21], B16 murine melanoma [Bergeron et al., submitted for publication], and oncogene-transfected Rat-1 cell lines [10]. As summarized by Bernacki et al. [5], among the analogues tested to date, BENSPM exerts the greatest in vivo activity.

BENSPM displays interesting pharmacologic activity in that it induces striking (several hundred-fold) elevations of spermidine/spermine N^1-acetyltransferase (SSAT) activity. Although it is tempting to attribute its slightly superior in vitro antiproliferative activity to its ability to induce SSAT, an imperfect correlation of its SSAT induction with its antiproliferative activity exists [12], and the order of

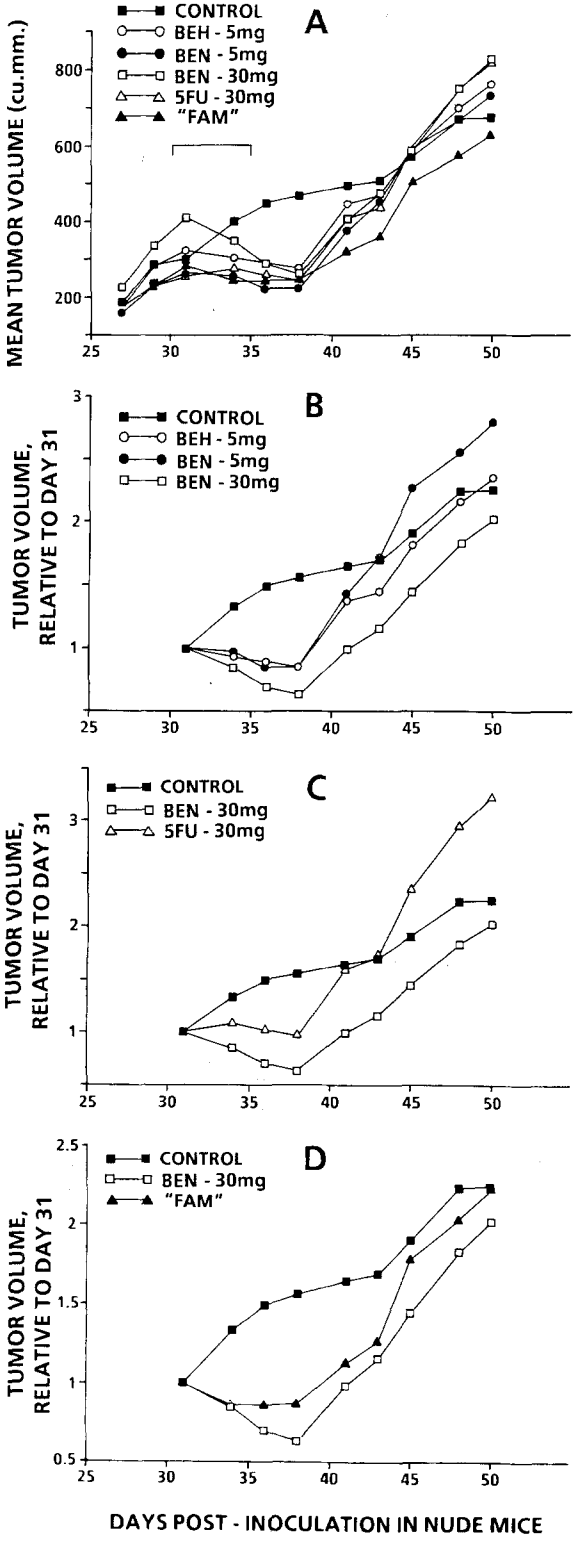

Fig. 2 A–D. Comparison of the in vivo antitumor effects of BEHSPM, BENSPM (at 2 dose levels) 5FU, and FAM against athymic (nude) mouse xenografts of BxPC-3. (See Table 1 for details of the therapy.) Treatment extended from day 30 through day 35 as indicated by the *bar* in **A**. **A** Actual tumor volumes and growth curves for all groups ($n = 10$ animals each). **B–D** Tumor volumes relative to those measured on day 31. **B** Effect of BENSPM as compared with BEHSPM. **C** Effect of BENSPM as compared with 5FU. **D** Effect of BENSPM as compared with FAM

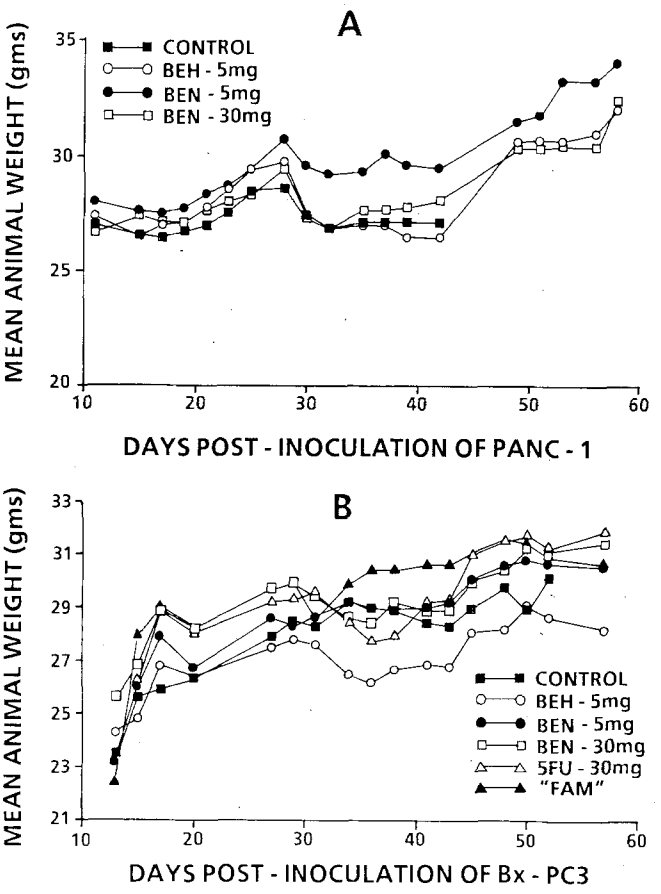

Fig. 3 A, B. Effects of BEHSPM and BENSPM on the body weight of athymic (nude) mice. **A** Mice bearing xenografts of PANC-1; these animals were treated from day 27 through day 32. (See Table 1 for details of the therapy). **B** Mean weights of athymic (nude) mice bearing xenografts of BxPC-3; these animals were treated from day 30 through day 35. No weight loss observed in any treatment group was significantly greater than that seen in untreated control animals. There was no drug-related mortality

magnitude of the differences argues against the hypothesis that its induction of SSAT plays a major role in its in vivo activity. As seen in the present study, at similar, roughly equimolar doses of BEHSPM and BENSPM (5 mg/kg doses), the resultant antitumor activity was quite similar. The major difference in the two analogues appears to be that of host toxicity, which enabled the administration of much larger doses of BENSPM, which seemed to show some preferential cytotoxicity for the pancreatic cancer xenografts. Recently, Bernacki et al. [5] treated human melanoma MALME-3 xenografts with 40 to 80 mg/kg doses given three times daily for 6 days and found that a tumor-growth delay of 46 days occurred at either dose. These authors estimated that the maximal individual dose on the same schedule might be as high as 120 mg/kg. At the time our experiments were performed, 30 mg/kg doses given for 6 days was felt to represent a nearly maximal dose of BENSPM. Clearly, nude mice could have tolerated higher doses, and at this point, one can only speculate as to the improvement in antitumor response that might have been obtained using higher doses. Thus, optimal doses and schedules of administration of BENSPM remain to be determined.

Table 1. Assessment of antitumor effects of *N*-alkylated polyamine analogues in xenografts of human pancreatic adenocarcinoma

Treatment and response assessment	Tumor model	
	PANC-1	BxPC-3
Control tumor-doubling time	4.5 ± 2 days	6.0 ± 1.4 days
BENSPM, 30 mg/kg doses:[a]		
% Tumor regression[b]	33.0%	36.7%
Growth delay	14 days	5 days
Estimated log cell kill[c]	0.94	0.25
BENSPM, 5 mg/kg doses:		
% Tumor regression	2.7%	15.2%
Growth delay	7 days	5 days
Estimated log cell kill	0.47	0.25
BEHSPM, 5 mg/kg doses:		
% Tumor regression	0	14.9%
Growth delay	8 days	5 days
Estimated log cell kill	0.54	0.25
5FU, 30 mg/kg daily:		
% Tumor regression	NA	11.21%
Growth delay		6 days
Estimated log cell kill		0.30
FAM:[a]		
% Tumor regression	NA	13.7%
Growth delay		6 days
Estimated log cell kill		0.30

[a] All *N*-alkylated polyamine analogues were given i.p. every 8 h for 6 days; 5FU was given once daily for 6 days; and FAM treatment consisted of 20 mg/kg 5FU, given on days 30, 37, 57, and 64; 2.5 mg/kg Adriamycin (doxorubicin) given on days 30 and 57; and 3.0 mg/kg mitomycin C given on day 30 only. Controls received a comparable volume of normal saline. Control and treatment groups comprised 10 animals each

[b] % Tumor Regression represents the average percentage of decrease in tumor volume from the largest pretreatment value

[c] Estimated log cell kill represents the estimate of log cell kill based on growth delay as determined using the method of Corbett et al. [14], which takes into account the tumor-doubling time

NA, Not applicable

Nevertheless, the studies reported support the conclusion that BENSPM is an active agent in xenografts of human pancreatic adenocarcinoma, a disease for which effective therapy is sorely lacking. Indeed, the activity demonstrated to date for BENSPM in human lung cancer, melanoma, and pancreatic cancer – all relatively chemoresistant solid adult tumors – makes it an attractive agent for further development.

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