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ORIGINAL ARTICLE

**Benjamin Frydman · Carl W. Porter
Yulia Maxuitenko · Aparajita Sarkar
Subhra Bhattacharya · Aldonia Valasinas
Venodhar K. Reddy · Nick Kisiel
Laurence J. Marton · Hirak S. Basu**

A novel polyamine analog (SL-11093) inhibits growth of human prostate tumor xenografts in nude mice

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Abstract *Purpose*: We tested the polyamine analog SL-11093 (3,8,13,18-tetraaza-10,11-[(E)-1,2-cyclopropyl] eicosane tetrahydrochloride) as an effective chemotherapeutic agent against human prostate cancer grown in nude mice. *Methods*: NCr-nu mice grafted with DU-145 human prostate tumor cells were treated i.p. with SL-11093 at 50 mg/kg q1d×5 for either three or five cycles separated by intervals of about 10–15 days. *Results*: In treated animals, tumor growth remained arrested for up to 100 days with minimal animal weight loss. None of the animals died during the treatment and in one experiment two out of six animals showed no palpable tumor. SL-11093 was readily taken up by the tumors, where its levels remained elevated for about 48 h after the end of drug administration. In liver and in kidney, SL-11093 (a $^{\alpha}$N,$^{\omega}$N-bisethyl derivative) was oxidatively N-deethylated predominantly to its monoethyl and di-deethyl derivatives. In time, the monoethyl derivative was further dealkylated, with a loss of an aminobutyl chain to form an aminomethyl cyclopropyl derivative. In tumor (and in lung), N-dealkylation reactions were less evident. *Conclusion*: SL-11093 is an effective chemotherapeutic agent against a human prostate tumor xenograft grown in nude mice. The drug accumulation and slow metabolism in tumor compared to other tissues would most likely reduce systemic toxicity of the drug and contribute to a larger therapeutic window for SL-11093 as compared to other cytotoxic polyamine analogs.

Keywords Polyamine analog · Human prostate tumor · Nude mouse xenograft · Metabolism · Antitumor effect

B. Frydman · A. Sarkar · S. Bhattacharya · A. Valasinas
V. K. Reddy · L. J. Marton · H. S. Basu (✉)
SLIL Biomedical Corporation,
Suite C, 535 Science Dr, Madison,
WI 53711-1066, USA
E-mail: hsbasu@slil.com
Tel.: +1-608-2313875
Fax: +1-608-2313892

C. W. Porter · N. Kisiel
Grace Cancer Drug Center,
Roswell Park Cancer Institute,
Buffalo, NY 14263, USA

Y. Maxuitenko
Southern Research Institute,
Birmingham, AL 35255-5305, USA

Introduction

Prostate cancer is the second most common cancer in American males, following lung cancer. This year (2003), it is estimated that about 220,900 new cases of prostate cancer will be detected in the US, and that about 28,900 men will die of this disease [1]. All rapidly proliferating and highly undifferentiated cancer cells, including human prostate cancer cells, synthesize and accumulate the polyamines spermidine and spermine and tumor-bearing animals and patients have elevated levels of polyamines in their extracellular fluids [6]. The role of polyamines in cell division, in maintaining the fidelity of protein synthesis and the potential usefulness of polyamine analogs as antiproliferative agents against many tumor cell lines have been extensively discussed elsewhere [12, 18]. Of the many theories advanced to explain the biological effects of the polyamines, the hypothesis centering on their binding to nucleic acids is the most compelling one [7]. Both spermine and spermidine are polycations that bind to the negatively charged nucleic acids, induce changes in the secondary structure of DNA and alter the flexibility of the DNA and t-RNA chains [11, 14]. $^{\alpha}$N,$^{\omega}$N-Bisethyl spermine and its higher and lower homologs bind regioselectively to t-RNA because of the different hydrogen-bonding modes that are formed between the molecules [13]. We therefore synthesized conformationally rigid analogs of

the flexible spermine derivatives with the expectation that their binding to DNA and/or t-RNA would affect nucleic acid topology and thereby inhibit protein and DNA synthesis and cell proliferation [19, 20, 22]. The semen of healthy men contains large amounts of spermine (about 3 m*M*) that originates mainly from prostatic secretion [23]. Thus it is perhaps not surprising that human prostate cancer cell lines are in general sensitive to polyamine analogs and polyamine biosynthesis inhibitors.

The conformationally restricted $^{\alpha}$N,$^{\omega}$N-bisethylspermine analogs, as well as their higher tetramine and pentamine homologs, are markedly cytotoxic against cultured human prostate cancer cell lines [19, 20, 22]. As is the case with most anticancer drugs [4, 5], these analogs are not devoid of systemic toxicity and their cytotoxicity must be balanced with their toxic side effects. A series of preliminary MTD (maximum tolerated dose) studies in animals helped select one of the least toxic among them: the $^{\alpha}$N,$^{\omega}$N-bisethyl polyamine analog SL-11093 (3,8,13,18-tetraaza-10,11-[(E)-1,2-cyclopropyl]eicosane tetrahydrochloride). This analog strongly inhibited the growth of cultured human prostate tumor cell lines [22]. Among four prostate tumor lines tested, DU-145 cells showed the most sensitivity to this analog. We report here that when assayed in vivo in DU-145 tumors xenografted in nude mice, SL-11093 also showed a strong inhibition of tumor growth at doses that had minimal systemic toxicity in the animals.

Materials and methods

Materials

SL-11093 was obtained by total synthesis [22]. Spermine and spermidine were purchased from Calbiochem-Novabiochem Corporation (La Jolla, Calif.). Putrescine and dansyl chloride were purchased from Sigma (St. Louis, Mo.). Sterile Dulbecco's phosphate-buffered saline was purchased from GibcoBRL Life Technologies (Grand Island, N.Y.). All reagents were of reagent grade and deionized water was used as solvent.

Nude mouse xenograft

The DU-145 human prostate tumor was maintained either in culture or by continuous in vivo passage in male athymic NCr-nu mice. Mice were housed in microisolator cages (five per cage) on a 12-h light/dark cycle in a barrier facility. Either tumor cell suspension (1×10^6) or tumor fragments (about 2×3 mm) were implanted subcutaneously with a 12-gauge trocar needle. Tumor size was measured twice per week in two perpendicular dimensions with a vernier caliper and converted to tumor volume using the formula: $(l\times w^2)/2$, where l and w refer to the longer and shorter dimensions, respectively. Animal body weights were measured twice per week at the same times as the tumor dimension measurement and mortality was monitored daily. Effects of treatment on tumor growth, expressed as percent growth delay, were calculated following the relation: percent growth delay = $[(T-C)/C]\times100\%$, where T and C represent the median times post-staging for treated (T) and control (C) tumors to attain a prescribed size depending on the model. SL-11093 was administered intraperitoneally (i.p.) in water using a chronic treatment schedule: administration for several cycles of once a day for five consecutive days (q1d×5) with a rest period of about 10–15 days between each cycle. Treatments were initiated about 15 days after tumor implantation when the mice had established tumors ranging in size from 100 to 245 mm^3.

Metabolic studies

Twelve animals were treated with SL-11093 formulated in water for injection. The animals were injected i.p. in a single cycle of q1d×5 at a dosage of 50 mg/kg/dose. Two animals received vehicle (water for injection) alone. Blood was collected from two mice in the treated groups at 1, 6, 24, 48, and 96 h after the last dose. Two animals in the control group were bled 6 h after the last injection. Blood was collected and the plasma fraction was separated and frozen. Animals were killed and tumor, liver, kidneys and lungs were collected and frozen in liquid nitrogen. Plasma and tissue samples were stored at −80°C until analyzed.

Tissue analysis

Tumor tissues and other organs were homogenized in 1 ml PBS using a Power Gen-700 homogenizer and centrifuged at 10,000 rpm for 10 min at 4°C. After centrifugation, the pellet was resuspended in 0.5 ml 2% perchloric acid and stored overnight at −20°C. The pellet was sonicated with three 30-s pulses and then centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was collected and its polyamine composition was analyzed by derivatization with dansyl chloride followed by HPLC separation as described elsewhere [15]. Each dansyl-polyamine derivative was collected and its molecular weight was determined by mass spectrometry (MS-ESI).

Enzyme assay

The enzymes ODC, S-adenosylmethionine decarboxylase (S-AdoMetDC) and spermidine/spermine N^1-acetyl transferase (SSAT) in cultured DU-145 cells were assayed following published procedures [9, 17, 21].

Results

In the first experiment, the treatment was initiated against established tumors (100–245 mm^3) at analog doses near the MTD (50 mg/kg once daily for 5 days, q1d×5) as determined in normal mice (see Methods). This 5-day treatment was repeated three times with a rest period of about 15 days between each cycle. A plot of tumor volume versus time is shown in Fig. 1A. Treatment with SL-11093 produced a growth delay of $>386\%$, and the T/C ratios were 52%, 34%, and 25% after the end of the first, second, and third cycles of treatment, respectively. Moreover, two out of six animals were apparently tumor-free at the termination of the experiment. Body weight loss at the end of the experiment was about 11%. Because there was minimal host weight loss and no evidence of toxicity, we evaluated SL-11093 in other experiments in which the 5-day dosing schedule (q1d×5) was repeated for five cycles with a rest period of about 10 days between the cycles against tumors that were larger than those in the previous experiment. A plot of these tumor volumes versus time is shown in Fig. 1B. The growth of tumors in treated mice

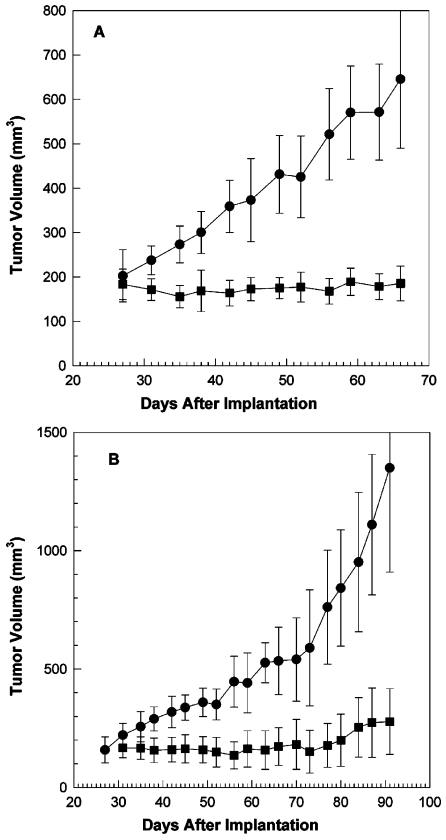


Fig. 1A, B Effect of SL-11093 (50 mg/kg, q1dx5) on DU-145 human prostate tumor growth in nude mouse xenografts. **A** Experiment with three cycles of drug administration over 68 days (Southern Research Institute, Birmingham, Ala.): days 12–16, days 31–35, and days 52–56 after tumor implantation. Each data point is the average from six mice (*n*) each in the control (●) and the drug-treated group (■). **B** Experiment with five cycles of drug administration over 94 days (Roswell Park, Buffalo, NY): days 28–32, days 44–48, days 58–62, days 72–76, and days 84–88 after tumor implantation. Each data point is the average of six mice (*n*) each in the control (●) and the drug-treated group (■)

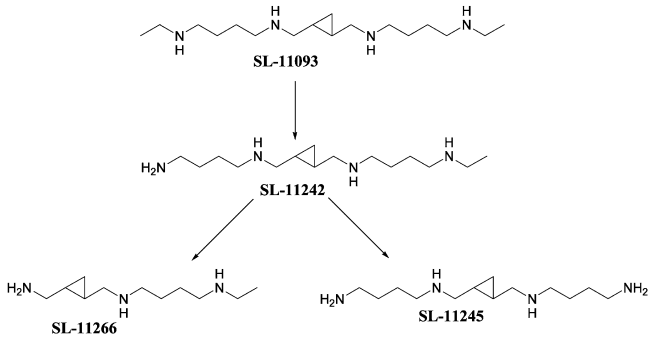
was again markedly suppressed relative to that in saline-treated control animals. Tumor growth remained relatively flat for about 100 days and resumed slowly about 20 days following the last round of treatment. Saline-treated mice gained weight (from 31 g to 37 g) during the 100-day experiment, while the weight of the SL-11093 treated mice remained essentially unchanged (from 31 g to 32 g) during the treatment. Taken together, both experiments confirmed that multiple cycles of treatment with SL-11093 produced meaningful antitumor responses in human prostate tumor xenografts in animals with minimal host toxicity.

The metabolic profile of SL-11093 was determined in male athymic NCr-nu mice implanted subcutaneously with DU-145 tumors (see Methods). The metabolic pathway for SL-11093 is shown in Scheme 1. The N-ethyl substituents were sequentially cleaved by oxidative dealkylation reactions to give mainly the deethylated polyamines SL-11242 and SL-11245. A third minor component of the metabolite mixture was SL-11266, which was formed by cleavage of the aminobutane chain. The identities of the metabolites were established by comparing the analytical and chromatographic data obtained from animal samples with those of synthetic samples.

The serum clearance profile for SL-11093 showed a steep decline: 12 h after the last injection, SL-11093 was undetectable in serum (Fig. 2A). In contrast, SL-11093 was readily taken up by the tumor, where its levels stayed high for about 48 h after the end of drug administration (Fig. 2B). SL-11093 was not found to be deethylated in the tumor to any appreciable degree during the 48 h following the last dosing, a fact that most likely contributed to its cytotoxic effect. In liver, the drug was efficiently oxidized mainly to its di-deethyl derivative SL-11245 (Fig. 2C). After 48 h, SL-11093 levels were markedly decreased and SL-11245 was its major metabolic product, while SL-11242 was the minor metabolite and SL-11266 was present in very small amounts. In kidney, both the intact drug and its main metabolite (SL-11245) were present at high levels, while SL-11242 was again the minor metabolite with SL-11266 present in very small amounts (Fig. 2D). Interestingly, SL-11093 was also readily taken up by lung, where it did not undergo extensive degradation (Fig. 2E). Its effect against lung tumors is currently under investigation.

It can be concluded that SL-11093 is a polyamine analog that accumulates in DU-145 human prostate tumor xenografts, where it remained at a high levels during the time period studied (96 h) without appreciable metabolic degradation. During this period, it exerted its cytotoxic effect and significantly inhibited tumor growth. In liver and kidney as well as in large and small intestine (data not shown), the diamine SL-11245 was the major metabolite of SL-11093 found to accumulate.

The effects of SL-11093 on the levels of the natural polyamines (spermine, spermidine, and putrescine) in specific tissues are shown in Fig. 3. In tumor, where there were periodic increases in the levels of putrescine in the untreated tissue, treatment with SL-11093 induced higher levels of putrescine than were present in untreated tumor. Although this suggests a possible inhibition of the S-AdoMetDC enzyme or an induction of the salvage pathway enzyme SSAT [12], no significant changes in the activities of these enzymes were found after treatment with SL-11093 (Table 1). The levels of spermine and spermidine in the tumor were not significantly affected by the drug. Treated animals had lower levels of spermidine and spermine, and higher levels of putrescine, in the liver than untreated controls. Untreated animals had higher levels of spermine and putrescine, and lower levels of spermidine, in the kidney than treated animals. Both spermine and spermidine levels decreased with treatment, while putrescine levels remained high. In lung, spermine and spermidine levels were not significantly affected by treatment with SL-11093, but putrescine levels were strongly increased. This is similar to what occurred in the tumor. In both



Scheme 1 Metabolic pathway for SL-11093

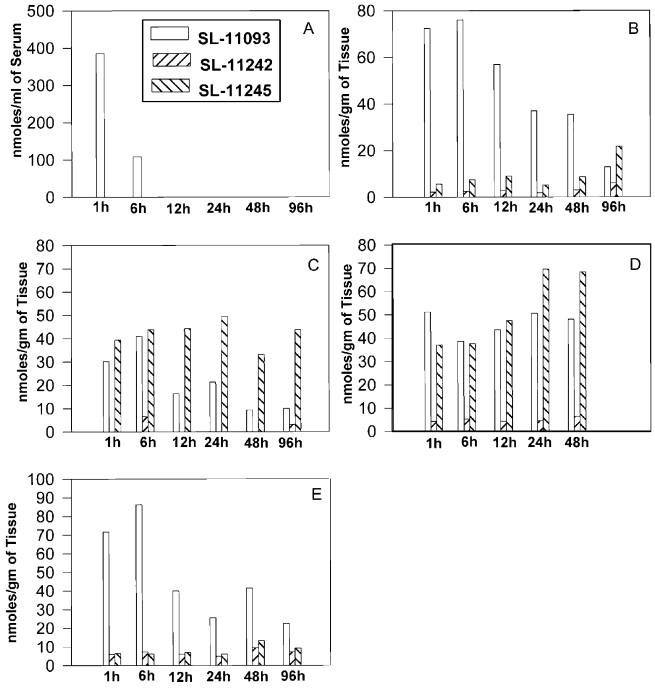


Fig. 2A–E Levels of SL-11093, monoethyl-SL-11093 (SL-11242) and deethyl-SL-11093 (SL-11245) in serum (**A**), tumor (**B**), liver (**C**), kidney (**D**) and lung (**E**) tissues harvested 1, 6, 12, 24, 48, and 96 h after the last day of i.p. administration of SL-11093 (q1dx5)

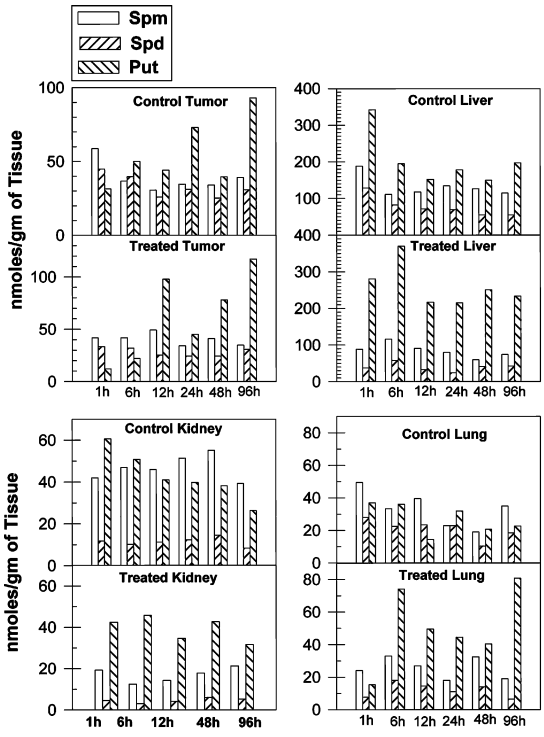


Fig. 3 Levels of putrescine, spermidine and spermine in tumor, liver, kidney and lung tissues measured 1, 6, 12, 24, 48, and 96 h after the last day of i.p. administration of 50 mg/kg SL-11093 (q1dx5)

Table 1 Effect of SL-11093 on the activities of ODC, S-AdoMetDC and SSAT in cultured DU-145 cells

SL-11093 concentration (μM)	Enzyme (pmol/h/mg protein)		
	ODC	S-AdoMetDC	SSAT
0	12.21	1.14	90
1	11.77	2.17	160
10	0.49	1.06	123

tumor and lung, high levels of SL-11093 persisted for longer times than in other tissues studied without deethylation to its metabolites. It appears that higher SL-11093 levels in these tissues correlated with an increase in putrescine levels. This increase in putrescine level, however, could not be attributed to the induction of the biosynthetic enzyme ornithine decarboxylase (ODC), as SL-11093 is an inhibitor of ODC activity (Table 1). The ability of SL-11093 to induce cellular polyamine uptake from serum has not yet been tested.

Discussion

Recently, there has been an upsurge in the search for chemotherapeutic agents capable of inhibiting prostate tumor growth. The quest for drugs with low systemic toxicities resulted in the crafting of prodrugs in which peptide sequences were conjugated with an established cytotoxic drug such as doxorubicin. These conjugates were specifically hydrolyzed by the proteolytic enzyme prostate specific antigen (PSA) with the release of the drug at tumor sites [8, 16]. Although the peptide conjugates were less toxic than doxorubicin itself, a possible problem with this approach is that, while several prostate tumor tissues secret PSA, many others (including DU-145) do not [10]. Additionally, the successful treatment of metastatic tumors with peptide conjugates has not yet been ascertained. It is, therefore, timely to develop a new low molecular weight drug such as SL-11093 that has excellent aqueous solubility and that acts on prostate tumors independently of their secretion of tumor antigens. SL-11093 appeared to have minimal systemic toxicity, was well tolerated, inhibited prostate

tumor growth by about 80% when given to athymic mice grafted with the human prostate tumor DU-145 and exerted its effect even when administered to animals that had already developed well-established tumors of about 200 mm^3.

Noteworthy is the relatively low metabolic N-deethylation of SL-11093 in the tumor. It has been suggested that the metabolic N-deethylation of bis(ethyl)polyamine analogs to primary amines is a major cause of an analog's systemic toxicity [4, 5]. The fact that SL-11093 stays mostly intact in the tumor most likely contributes to its cytotoxic effect. Its major metabolite SL-11245 (Scheme 1) is a much weaker inhibitor of DU-145 cell growth in culture (the IC_{50} of SL-11093 is 0.06 μM, while the IC_{50} of SL-11245 is 0.60 μM). We have determined that the oxidative dealkylation of SL-11093 is not the result of the action of cytochrome P450 isozymes or of diamine oxidase (data not shown). Oxidative dealkylation is more likely to be attributed to the action of a polyamine oxidase (PAO) [18], an enzymatic reaction we are currently exploring. Of interest is the presence of SL-11266 among the metabolites (Scheme 1), since it has been reported that the polyamine analogs that carry an N-ethyl butylamine chain are not degraded by the PAO/SSAT system, while only analogs carrying N-ethyl propylamine chains are [4, 5]. The presence of very small amounts of SL-11266 and of its most likely precursor SL-11242 in liver and kidney suggests the presence of an active oxidase in these organs; alternatively, the metabolites may be substrates of an efficient excretory pathway.

Similar to other cytotoxic polyamine analogs [2], SL-11093 interacts with DNA and has a marked effect on decondensing cellular chromatin. For some analogs this action may affect the expression of specific genes [3]. The low systemic toxicity of SL-11093 could be due to its ability to inhibit the expression of specific cell cycle gene(s) in prostate tumor. This can be a distinct endpoint for measuring the therapeutic effectiveness of SL-11093, which is currently being investigated as a prelude to forthcoming clinical trials.

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