Cancer Chemother Pharmacol (1999) 43: 198–204

ORIGINAL ARTICLE

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Efficacy of suramin against human prostate carcinoma DU145 xenografts in nude mice

Received: 9 March 1998 / Accepted: 29 June 1998

Abstract *Purpose*: Toward developing a model to study the mechanism of action of suramin against prostate cancer, we identified the effect of suramin on the growth of xenografts of the androgen-independent human prostate carcinoma DU145 cell line and our subline of suramin-resistant (SR) DU145 cells which are less responsive to suramin in vitro. *Methods*: Athymic nude mice bearing DU145 or SR DU145 xenografts were treated intraperitoneally (IP) once weekly with normal saline (vehicle control) or suramin in normal saline. For data analysis mice were grouped as follows: 0 mg/kg (controls), < 210 mg/kg, 210 to 260 mg/kg, or > 260 mg/kg suramin. *Results*: The growth of DU145 xenografts was slowed by treatment with 210 to 260 mg/kg suramin IP once weekly: differences in tumor volume for the 210 to 260 mg/kg group compared with the control group on days 29 and 57 showed growth inhibited by 43% and 55%, respectively. At the same time, growth of SR DU145 xenografts generally was not slowed by suramin treatment at any dose, but appeared to be enhanced to some degree by all doses of suramin during the typical slower initial growth phase of xenografts of this cell line: differences in tumor volume compared with control on day 29 showed growth enhanced by 100% to 342%. Mice treated with 210 to 260 mg/kg maintained nadir suramin plasma levels near our clinically relevant target of 1×10^{-4} *M*. *Conclusions*: Suramin, without concomitant corticosteroid therapy, was effective in slowing the growth of DU145 xenografts in nude mice at clinically relevant plasma suramin levels. The data showing efficacy for DU145 xenografts was supported by the lack of efficacy at the same time for xenografts of cells known to be less responsive to suramin in vitro, i.e. the SR DU145 cells, at similar doses and nadir plasma suramin levels. In discussions on the utility of suramin our data should be considered as support for continuing the study of suramin in the treatment of advanced, androgen-independent prostate cancer.

Key words Suramin · Prostate cancer · Nude mice

This work was supported by NIH/NCI RO1 CA57456

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Introduction

It is estimated for the United States that one in five men will develop invasive prostate cancer within their lifetime and in 1998 alone about 39 000 men will die of prostate cancer. These estimates place prostate cancer among the highest for cancer incidence and mortality for US men [10].

Though greater than 75% of newly diagnosed metastatic prostate cancer patients have at least in part androgen-dependent disease responsive to androgen therapy, virtually all metastatic prostate cancer patients eventually develop androgen-independent disease [26]. There is no standard treatment for advanced, androgen-independent prostate cancer [8]. Suramin is a novel agent which has shown promise for the treatment of prostate cancer [3, 4, 8, 21], but the efficacy of suramin alone remains controversial. There have been reports of limited efficacy of suramin in the treatment of hormone-refractory prostate cancer clinically [18] and lack of efficacy of suramin against some androgen-independent human prostate carcinoma xenografts [17, 23]. Suggestions that the efficacy of suramin in the treatment of prostate cancer is in part due to the concomitant

hydrocortisone therapy prompted a phase III study of suramin with hydrocortisone versus a placebo with hydrocortisone for metastatic hormone-refractory prostate cancer. A recent report of the results of this study at a median follow-up of 21 months show that suramin plus hydrocortisone has a significant palliative advantage and produces a delay in disease progression [20]. These results will play a role in deciding the utility of suramin in the treatment of prostate cancer.

The mechanism of action of suramin has not been clearly established [3, 4, 21]. In developing a model to study the mechanism of action of suramin against prostate cancer, we tested the effect of suramin, without concomitant corticosteroid therapy, on the growth of xenografts of the androgen-independent human prostate carcinoma DU145 cell line in nude mice. We simultaneously tested the effect of suramin on xenografts of our suramin-resistant (SR) DU145 cell line. The SR DU145 cell line was established in our laboratory by continual culturing in medium containing 0.4 m*M* suramin, and is fivefold less responsive to suramin in vitro [16]. We report that suramin alone was effective in slowing the growth of DU145 xenografts at clinically relevant plasma suramin levels. The data showing efficacy for DU145 xenografts were supported by the lack of efficacy at the same time for SR DU145 xenografts at similar doses and nadir plasma suramin levels. Our data support continued study of suramin in the treatment of advanced, androgen-independent prostate cancer.

Materials and methods

Materials

The established human prostate carcinoma cell line DU145 was obtained from the American Type Culture Collection (Rockville, Md.). The SR DU145 cell line was established in our laboratory by continual culturing of DU145 cells in medium containing 0.4 m*M* suramin. Suramin was purchased from FBA Pharmaceuticals (New York, N.Y.).

Cell culture

Cells were grown at 37 °C under humidified air containing 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% heat-inactivated fetal bovine serum (both GIBCO BRL products, Grand Island, N.Y.) and 1% antibiotic antimycotic solution (100X) (Sigma). The medium for SR DU145 cells additionally contained 0.4 m*M* suramin. Cells were passaged weekly and the medium was replaced every 3 to 4 days. For preparations of cell suspensions for subcutaneous injection, cells were harvested by trypsinization (Trypsin-EDTA, GIBCO BRL, Grand Island, N.Y.), resuspended in DMEM, and counted on a standard hemacytometer.

Animals

Male Hsd: athymic nude-*nu* (*nu*/*nu*, BALB/c origin) mice at 3 to 4 weeks of age were purchased from Harlan Sprague Dawley (Madison, Wis.) and allowed to acclimate for 2 weeks prior to manipulation at 5 to 6 weeks of age. The mice were housed under laminar air flow in an isolated animal unit in which the animal rooms were maintained at 25–28 °C and 60–70% humidity with 12 h light and dark cycles. A maximum of four mice were housed per autoclave-sterilized cage with free access to autoclave-sterilized water and food. Mice bearing tumors were euthanized when the tumor burden reached 10 to 15% of body weight. Mice obviously ill prior to reaching this level of tumor burden were euthanized immediately. For all mice the euthanasia procedure consisted of: anesthetization with etomidate (Amidate, Abbot Laboratories, North Chicago, Ill.) administered at 25 to 30 mg/kg intraperitoneally (IP), blood collection by retro-orbital puncture for plasma harvest, then cervical dislocation and necropsy. Manipulation of mice was in accordance with a protocol approved by the CHS Animal Care Committee of the University of Wisconsin-Madison, USA. Plasma and sections of tumors and select tissue samples collected at necropsy were stored at −80 °C for future analysis.

Experimental design

Two studies were performed to determine the efficacy of suramin for DU145 and SR DU145 xenografts in nude mice. For each cell line in both studies 1 and 2, each of 16 mice received subcutaneous injections of 1×10^6 cells in 0.1 ml DMEM in the vicinity of two separate ventral fat pads. Body weight and tumor size were measured at least once 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 [5]: length × width^2 (in millimeters)/2 = volume (in cubic millimeters).

In both studies suramin treatment was initiated 8 days following cell injection. For each cell line, mice were separated into four groups of four to receive IP injections of 0.1 ml sterile 0.15 *M* NaCl (controls) or 3.6, 5.4, or 7.2 mg suramin in 0.1 ml sterile 0.15 *M* NaCl once weekly. In the first study, nontumor-bearing mice of the same age were also treated once with 0.1 ml sterile 0.15 *M* NaCl (controls) or 1.8, 3.6, 5.4, or 7.2 mg suramin in 0.1 ml sterile 0.15 *M* NaCl and sacrificed 2 or 21 h later (n = four mice per treatment group, two sacrificed at each time-point).

Blood samples were collected by retro-orbital puncture under etomidate anesthesia from the nontumor-bearing mice for assessment of plasma suramin levels achieved within 24 h of administration and from xenografted mice for assessment of nadir suramin levels biweekly beginning with the first treatment in study 1 or for the third treatment only in study 2. Samples for nadir suramin levels were collected approximately 6 h prior to the next suramin treatment. Plasma was harvested from the blood samples and stored frozen at −80 °C until assay for suramin content.

Suramin assay

Plasma and tissue suramin concentrations were assayed by a reverse-phase ion-pairing high-performance liquid chromatography (HPLC) method [6, 7, 9, 22]. The extraction method used by Hutson et al. [6, 7] was modified for plasma to accommodate lower volumes of 50 to 100 µl plasma (extraction reagent volumes were reduced proportionately and dried extracts were dissolved in 0.25 to 0.5 ml mobile phase for HPLC analysis), while the extraction method for tissues [7] was used unmodified despite lower tissue wet weights of approximately 50 to 100 mg. The mobile phase was pumped at 1.8 ml/min through a NovaPak 4-µm C_{18} RadialPak 8 × 10 cartridge (Millipore Corporation – Waters Chromatography, Marlborough, Mass.).

Data analysis

Combination of studies

After studies 1 and 2 were completed, actual doses of suramin administered were calculated for each mouse by dividing the dose administered by body weight, and expressed as milligrams per kilogram. This accounted for a variation in body weights of mice between the studies and allowed us to combine the data from the

Table 1 Characteristics of groups of mice with DU145 xenografts compared with mice with SR DU145 xenografts. In two separate studies nude mice with DU145 xenografts or SR DU145 xenografts were treated with normal saline or suramin in normal saline IP once weekly beginning on day 8 of each study. Blood samples were collected for assessment of nadir plasma suramin levels biweekly beginning with the first treatment in study 1 or for the third treatment only in study 2. Plasma was harvested from the blood samples and extracted for HPLC analysis of suramin levels. For data analysis, mice in the two studies were grouped together based on their average actual dose of suramin from day 8 through day 57, rounded to the nearest 10 mg/kg, as follows: 0 mg/kg controls, <210 mg/kg, 210 to 260 mg/kg, >260 mg/kg

Actual suramin dose group		Range of actual suramin doses (mg/kg)	Range of nadir plasma suramin levels			
			First treatment nadir (day 15)		Third treatment nadir (day 29)	
mg/kg	*n*		*n*	(10^{-5} *M*)	*n*	(10^{-5} *M*)
DU145 mice						
0	8	0 (saline)	4	Negative	8	Negative
<210	10	111–204	4	2.51 to 3.73	9	2.51 to 7.64
210–260	9	213–251	3	5.06 to 8.51	9	4.65 to 10.6
>260	5	279–328	3	5.52 to 8.10	4	3.19 to 13.8
SR DU145 mice						
0	6	0 (saline)	3	Negative	8	Negative
<210	11	122–205	4	2.93 to 5.69	10	3.84 to 9.12
210–260	6	208–262	4	4.03 to 6.36	6	5.68 to 11.8
>260	6	279–358	3	10.4 to 17.5	6	1.90 to 14.1

two studies. For each xenograft cell line type, mice between the two studies were grouped together based on their average actual dose of suramin from the day 8 through the day 57 administrations, rounded to the nearest 10 mg/kg, as follows: 0 mg/kg controls, <210 mg/kg, 210 to 260 mg/kg, >260 mg/kg.

Statistics

The tumor volume and body weight data were analyzed using the method of analysis of repeated measurements. We confirmed that the usual assumptions for repeated measures designs were satisfied [12, 13]. (Note: the typical data point error margins were not needed for analysis using this model, since the data were correlated.) A two-tailed paired *t*-test was used to compare body weights of mice within a group at the start of treatment with subsequent days of the study. A confidence level of 0.05 was used for all analyses. Significant *P*-values are reported.

Results

The characteristics of the groups of mice with DU145 xenografts compared with mice with SR DU145 xenografts resulting from the combination across studies 1 and 2 were similar (Table 1). Mice bearing DU145 xenografts in the groups of <210, 210 to 260, and >260 mg/kg had day-8 through day-57 average actual doses ranging from 111 to 204, 213 to 251, and 279 to 328 mg/kg suramin respectively ($n = 10$, 9, and 5, respectively). The mice bearing SR DU145 xenografts in those groups had similar ranges of 122 to 205, 208 to 262, and 279 to 358 mg/kg suramin ($n = 11$, 6, and 6, respectively). Using 210 to 260 mg/kg as the target dose group proved consistent with our target of 1×10^{-4} *M* nadir plasma suramin levels: mice with either DU145 or SR DU145 xenografts in the 210 to 260 group had nadir suramin plasma levels generally falling within a half-log of the target 1×10^{-4} M with an overall range of 4.03 to 11.8×10^{-5} *M* for the first and third treatment nadir samples.

The growth rate of DU145 xenografts in the 210 to 260 mg/kg suramin dose group was reduced compared with that in controls, while the growth of DU145 xenografts in the surrounding dose groups, <210 and >260 mg/kg suramin, was similar to that in controls (Fig. 1A). Percent difference in tumor volume (TV) compared with controls on days 29 and 57, calculated by the equation percent difference in TV = [(mean TV suramin-treated/mean TV NaCl-treated)−1] × 100, showed DU145 xenograft growth inhibited by 43% on day 29 and 55% on day 57 for the 210 to 260 mg/kg group, and the mean TV of the controls and the 210 to 260 mg/kg groups on days 29 and 57 were significantly different ($P < 0.02$).

The growth of SR DU145 xenografts appeared enhanced by all suramin doses during the typical slower initial growth phase of xenografts of this cell line (Fig. 1C). For the dose groups of <210, 210 to 260, and >260 mg/kg, the day-29 differences in TV showed growth enhanced by 325%, 100%, and 342%, respectively, and the mean tumor volumes were significantly different compared with the controls for the 210 and >260 groups ($P = 0.02$, $P < 0.01$ respectively). At day 57 the <210 mg/kg group still showed moderate enhancement with a difference in TV of +30% compared with the controls, while the 210 to 260 and >260 mg/kg groups were not different from the controls. For all groups the mean TV was not significantly different from that of the controls on day 57.

By the repeated measurement model statistical analysis, average body weights were not significantly different among the groups for either the DU145 xenograft mice (Fig. 1B) or the SR DU145 xenograft mice (Fig. 1D). By the paired *t*-test statistical analysis, the body weights of mice with DU145 xenografts in the 210 to 260 mg/kg group from the start of treatment on day 8 to days 29 and 57 were not significantly different. Based on weekly

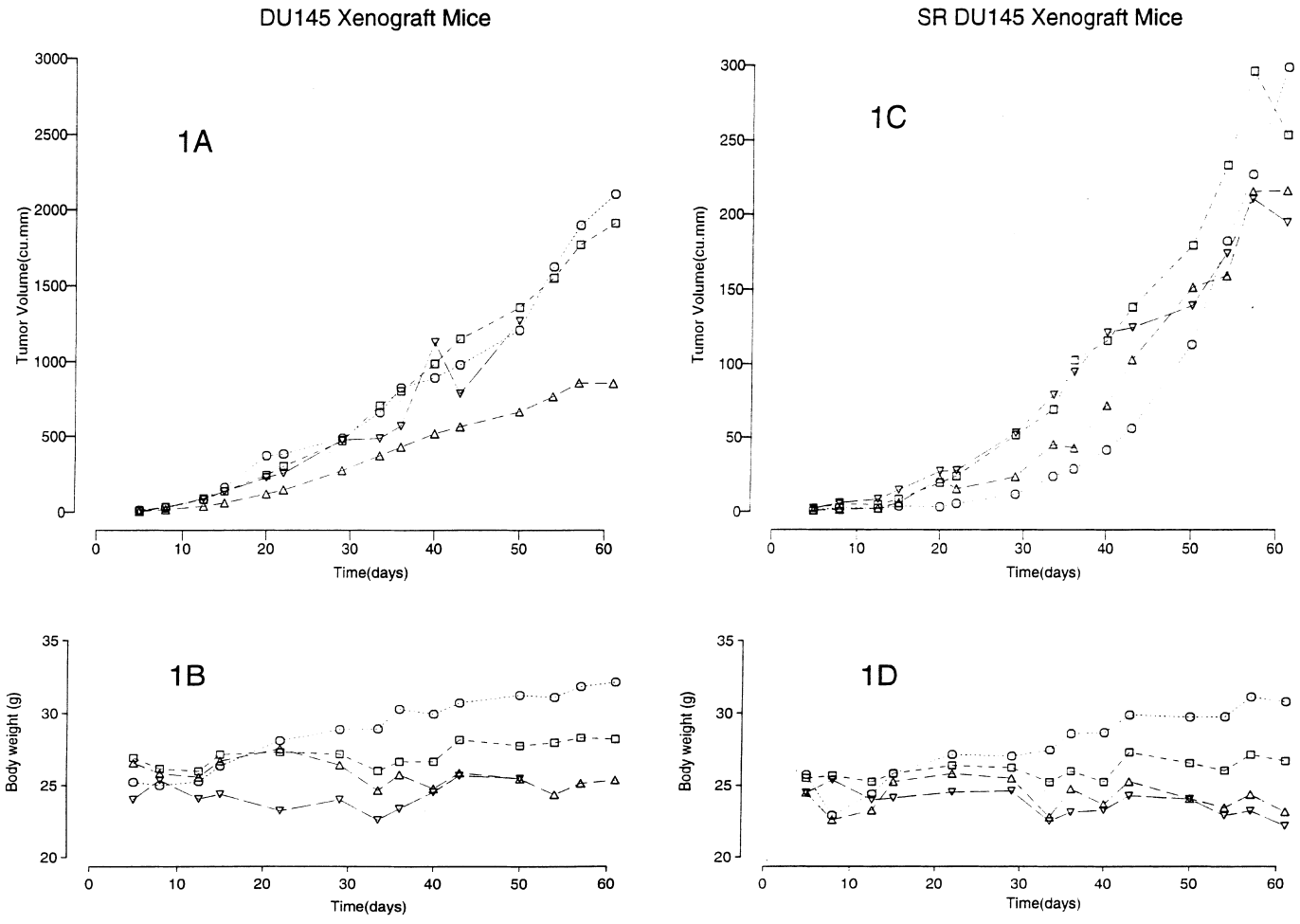

Fig. 1A–D Effect of suramin on growth of xenografts of human prostate carcinoma cell lines DU145 (**A, B**) and suramin resistant (SR) DU145 (**C, D**) in athymic nude mice. Cells were injected subcutaneously on day 0. Beginning on day 8 mice were treated IP once weekly with 0.15 *M* NaCl (vehicle control) or suramin in 0.15 *M* NaCl as follows: 0 mg/kg (controls, ○), < 210 mg/kg (), 210 to 260 mg/kg (), > 260 mg/kg (). Tumor sizes were measured at least once weekly and tumor volume was calculated by the equation: (length × width^2)/2 to compare tumor growth rates (**A, C** mean tumor volume, $n = 5$ to 19, with the exception of the > 260 mg/kg group for the DU145 cell line which had only two mice surviving past day 36, one bearing two tumors which survived through day 40 and one bearing one tumor which survived through day 50). Body weights of the mice were measured at least once weekly (**B, D** mean body weight, $n = 3$ to 11 also with the exception of the > 260 mg/kg group, as for **A, C**). Statistical analysis was done using the method of analysis of repeated measurements. Typical data point error margins were not needed for analysis using this model since the data were correlated. Under this model, comparison of suramin-treated groups with the respective control group for tumor volume data on days 29 and 57 showed significant growth inhibition for the DU145 210 to 260 mg/kg group for both days ($P < 0.02$), and significant growth enhancement for the SR DU145 210 mg/kg and > 260 mg/kg groups on day 29 ($P = 0.02$, $P < 0.01$ respectively). Body weights were not significantly different among the groups under the repeated measurements model. A paired *t*-test analysis of body weights at the start of treatment on day 8 to days 29 and 57 showed that the surviving mice in the SR DU145 > 260 mg/kg group had a significant reduction in body weight by day 57 ($P < 0.02$)

observations considering general appearance and tumor burden, the mice in the 5.4 mg group of study 1 and the 7.2 mg group of study 2, all but one of which were included in the 210 to 260 mg/kg grouping across the two studies, appeared in best overall condition when comparing groups of mice with DU145 xenografts. This was despite a skin pastiness and loss of skin turgor that appeared to be associated with suramin treatment at all doses for both DU145 and SR DU145 xenografted mice. For mice with DU145 xenografts, treatment at the > 260 mg/kg dose level was generally tolerated for three treatments with four out of five mice surviving on day 29 with body weights not significantly different from those on day 8, but continued treatment at this level of suramin proved intolerable with no survivors by day 51. Mice with SR DU145 xenografts similarly could not survive continued treatment at the > 260 mg/kg dose level: there were six out of six mice surviving on day 29 with body weights not significantly different from those on day 8, but by the eighth treatment (day 57) there were four surviving mice with significantly reduced body weights ($P < 0.02$), and these mice did not recover nor survive as long as mice in the other SR DU145 xenograft groups.

Analysis of plasma suramin levels in nontumor-bearing and DU145-xenografted mice indicated that all doses tested could achieve our target of 1×10^{-4} *M*, a

clinically achievable suramin plasma level. However, only actual doses averaging $\geq$210 mg/kg overall maintained the plasma levels within a half-log of the target (Table 2). The analysis of plasma levels in nontumor-bearing mice showed a dose response and all doses tested achieved greater than 1×10^{-4} *M* within 2 h, dropping by approximately half by 21 h (with the exception of two mice treated with 7.2 mg suramin, actual doses 321 and 329 mg/kg, which inexplicably were essentially negative for suramin in their plasma at 2 h; data not shown; Table 2.). Analysis of levels in xenografted mice suggested further reductions by another two to three halves by the first treatment nadir, approximately 162 h after administration (Table 2). A comparison between the first and the third treatment nadir plasma levels for the same three mice in each dose group (Table 2) suggested a lack of accumulation of suramin in the plasma after three treatments (by nadir time-point day 29) and showed that in the $<$210 mg/kg group suramin plasma levels were maintained at approximately 3×10^{-5} *M* while in the 210 to 260 and $>$260 mg/kg groups the levels were maintained at approximately 6×10^{-5} *M*. Lack of accumulation was generally true for all mice, comparing the first and third treatment nadir with sacrifice levels on an individual basis (data not shown). A sufficient number of mice in the $<$210 and the 210 to 260 mg/kg groups were sacrificed on a nadir day to compare suramin plasma and tumor levels between these groups (Table 2). These data show that suramin penetrated the tumors and indicates a correlation between nadir plasma levels and tumor levels in that mice with higher plasma levels also had higher tumor levels. This correlation appeared true for mice with SR DU145 xenografts as well (data not shown).

Discussion

In developing a model to study the mechanism of action of suramin against prostate cancer we needed to identify the effect of suramin on DU145 xenografts. Preliminary in vivo studies using suramin regimens based on those found effective for renal [2] and osteosarcoma [25] xenografts in nude mice have indicated that the respective suramin regimens of 3.6 mg IP once weekly or 1.8 mg IP on days 1, 3, 6, 9, and 14, then once weekly are not effective for DU145 xenografts and likely yielded nadir plasma suramin levels below our target of the clinically achievable 1×10^{-4} *M* level (data not shown). Subsequently, we designed study 1 to determine whether there were a higher dose suramin regimen that nadired near the target plasma suramin level and that was tolerable and effective against DU145 xenografts in nude mice. In that study we determined that a dose of 5.4 mg suramin IP once weekly met these requirements. We sought to confirm this result through a second study.

Table 2 Dose response and accumulation of suramin in plasma and DU145 xenograft tumors in nude mice. Nontumor-bearing nude mice were given a single IP administration of suramin then were sacrificed for blood collection 2 or 21 h later. From xenografted mice receiving suramin IP once weekly, blood was collected biweekly at nadir time points, i.e. approximately 162 h after the previous treatment (6 h prior to the scheduled weekly treatment), beginning with the first treatment nadir and also at sacrifice, and tumors were harvested at sacrifice as well. From the blood samples, plasma was harvested, extracted and analyzed by HPLC for suramin concentration. Tumors were sectioned and a portion was similarly extracted and analyzed by HPLC for suramin content. The reported first and third treatment nadir levels include only those for mice for which samples at both time-points were available. Reported sacrifice levels include only those for mice which were sacrificed at a nadir time-point

Time-point	Suramin dose (mg/kg)	Plasma suramin concentration		Tumor suramin concentration	
		n	(10^{-5} *M*)	*n*	μg/g
2 hours	80		14.4		
	96		16.2		
	152		28.5		
	163		30.3		
	230		52.1		
	239		43.8		
21 hours	87		5.29		
	90		6.21		
	140		10.0		
	149		10.1		
	189		17.6		
	244		22.6		
	319		20.1		
	330		33.7		
First treatment nadir	$<$210	3	2.87 ± 0.42		
	210–260	3	6.35 ± 1.88		
	$>$260	3	7.08 ± 1.37		
Third treatment nadir	$<$210	3	3.88 ± 0.67		
	210–260	3	6.26 ± 1.69		
	$>$260	3	5.77 ± 2.85		
At sacrifice (treatment nadir)	$<$210	5	4.89 ± 1.40	8	286.0 ± 70.2
	210–260	4	9.79 ± 1.60	6	393.4 ± 68.0

Study 2 was similar to study 1 but with a reduction to just one collection of blood for nadir plasma suramin level analysis to minimize stress on the mice induced by blood collection. In study 2 we confirmed that a dose of 5.4 mg suramin IP once weekly met the above requirements, but found that a dose of 7.2 mg suramin IP once weekly also met these requirements with the best overall health. Subsequent calculation of actual doses administered through day 57 clarified this difference in results between the two studies: the effective doses corresponded to average actual doses of 220 and 213 mg/kg in studies 1 and 2, respectively, for the 5.4 mg dose, and 251 mg/kg for the 7.2 mg dose in study 2, which was well below the 304 mg/kg for the 7.2 mg group in study 1 that was ineffective through the 3 weeks of treatment in which it was tolerable.

To combine the data from the two studies, we separated the mice into groups using the range of average actual doses which appeared to be effective, 210 to 260 mg/kg, as the basis for group assignment. Using this as a target dose group coincided with our clinically relevant plasma suramin level target, as mice assigned to the target dose group had nadir plasma suramin levels near our target 1×10^{-4} *M*. The studies together showed: (1) efficacy for mice with DU145 xenografts receiving once-weekly IP suramin at average actual doses in the range 210 to 260 mg/kg suramin and having clinically relevant nadir plasma suramin levels, (2) lack of efficacy for mice with DU145 xenografts receiving surrounding doses of once weekly IP suramin at average actual doses 210 or >260 mg/kg, despite the latter being tolerated and yielding similar nadir plasma suramin levels through three treatments, and (3) lack of tolerance for continued treatment with actual suramin doses averaging >260 mg/kg.

The findings were supported by the results from the concomitant study of mice bearing xenografts from our SR DU145 cell line. We established this SR DU145 line as a potential tool to study the mechanism of action of suramin in an in vitro/in vivo model of advanced, androgen-independent prostate cancer. Subsequent to demonstrating by proliferation assays that the SR DU145 cells are less responsive than DU145 cells to suramin in vitro, with the SR DU145 cells having a suramin IC_{50} dose of approximately 3×10^{-4} *M* which is five times greater than the suramin IC_{50} for the DU145 cells [16], we proceeded with these initial in vivo comparisons. Both in vivo studies together showed that the doses of suramin within the range that slowed the growth of DU145 xenografts, i.e. in the range of 210 to 260 mg/kg, at the same time were not effective in slowing the growth of the SR DU145 xenografts. Actually, growth appeared to be enhanced to some degree by all doses of suramin during the typical slower initial growth phase of the SR DU145 xenografts. However, as the growth rate of the control-treated SR DU145 xenografts approached that of the control-treated DU145 xenografts the data suggested a potential for inhibition of SR DU145 xenograft growth with suramin treatment. Indeed, data subsequent to the day-57 comparison (not shown), though statistically weak, supports this.

Two mice (four tumors between them) which were treated eight times with average actual doses on the low end of the <210 mg/kg group range (122 and 131 mg/kg) that we were able to observe through day 106 showed 62% inhibition when comparing tumor volumes with those of two control mice (three tumors between them) of like status (treated eight times and observed through day 106). Thus, suramin appeared to enhance SR DU145 xenograft growth during the typical slower initial growth phase but may have prevented the increase in growth rate subsequent to the initial slow growth phase. We have observed enhancement of growth with suramin in in vitro studies of primary prostate epithelial cells treated with 10^{-7} to 10^{-5} *M* suramin [14], and perhaps this can be related to the SR DU145 xenograft growth enhancement phenomenon. Since the analysis of SR DU145 tumors showed a correlation between plasma and tumor suramin levels similar to the findings in DU145 tumors, we can rule out lack of penetration of suramin into the SR DU145 tumors as a mechanism of resistance.

However, it is not the purpose of this report to explain the phenomena associated with the effect of suramin on SR DU145 xenograft growth. Further characterization of the SR DU145 cells is needed to draw conclusions on the apparent enhancement of early growth and the potential for inhibition of later growth of SR DU145 xenografts with suramin treatment. Rather, we wish mainly to illustrate that we observed lack of efficacy for xenografts of DU145 cells known to be less responsive to suramin in vitro, i.e. the SR DU145 cell line, during their early growth phase following transplantation from culture into nude mice. This lends credence to the apparent efficacy of suramin observed at the same time for the DU145 xenografts with similar suramin doses and nadir plasma suramin levels.

There are reports of the efficacy of suramin for an androgen-independent Dunning AT-2 rat prostate tumor [15] and as adjuvant therapy for Dunning AT-3 rat prostate tumor [19]. However, though there are reports of the efficacy of suramin for some types of human xenografts in nude mice such as osteosarcoma [25], renal carcinoma [2], colon carcinoma [11], hepatoma [1], and Wilms' tumor [24], we have found only reports of lack of efficacy of suramin against prostate cancer xenografts from androgen-independent cell lines PC-3 [17] and C4-2 [23]. We believe the results presented here are the first showing efficacy of suramin, at a clinically relevant plasma suramin level, for xenografts of an advanced, androgen-independent prostate cancer cell line in nude mice. In our studies we did not treat concomitantly with corticosteroid, so our data reflect the effect of suramin *alone* on the growth of DU145 xenografts. This is of importance since the effectiveness of suramin alone, i.e. without concomitant hydrocortisone therapy, for treatment of prostate cancer has been questioned. Our data suggest that suramin, without corticosteroid, can be

effective against prostate cancer. This is consistent with the results of the phase III trial of suramin with hydrocortisone versus a placebo with hydrocortisone for metastatic hormone-refractory prostate cancer for which it has been recently reported that suramin plus hydrocortisone is superior for palliative advantage and delay in disease progression [20].

As the utility of suramin is being decided, we offer our data as support for continuing the study of suramin in the treatment of advanced, androgen-independent prostate cancer. The development of in vitro/in vivo models in which the differences between suramin-sensitive cells and suramin-resistant cells can be characterized may lead us to a better understanding of the precise antineoplastic mechanism of action of suramin and thereby facilitate the development of more effective therapeutic strategies for this drug.

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