

Featured Article**Low-Dose Suramin Enhanced Paclitaxel Activity in Chemotherapy-Naïve and Paclitaxel-Pretreated Human Breast Xenograft Tumors**SaeHeum Song,¹ Bei Yu,¹ Yong Wei,¹
M. Guillaume Wientjes,^{1,2} and Jessie L.-S. Au^{1,2}¹College of Pharmacy and ²James Cancer Hospital and Solove Research Institute, The Ohio State University, Columbus, Ohio**ABSTRACT**

We reported induction of broad-spectrum chemoresistance by acidic and basic fibroblast growth factors and chemosensitization by their nonspecific inhibitor suramin at nontoxic and subtherapeutic doses. This study evaluated whether low-dose suramin enhances paclitaxel activity in chemotherapy-naïve and paclitaxel-pretreated human MCF7 breast xenograft tumors in mice. Suramin, 10 mg/kg, and/or paclitaxel, 15 mg/kg, were administered intravenously, twice weekly for 2 to 3 weeks. In addition to conventional end points [tumor size change, median survival time (MST)], we also used clinically relevant end points [partial (PR) and complete response rates (CR); progressive disease (PD); stable disease (SD); time to tumor progression (TTP)]. In chemotherapy-naïve mice, the control and suramin groups showed identical TTP (3 days) and MST (21 days). Single-agent paclitaxel produced 47% PR and 24% CR, and prolonged both TTP and MST to 73 days. The addition of suramin further improved the total response rate to 100% with a dramatically greater 63% CR, shortened the time to attain PR and CR, and prolonged TTP and MST to ≥ 136 days. In the paclitaxel-pretreated group, single-agent paclitaxel resulted in 67% SD and 33% PD, whereas the combination produced 50% PR and 50% SD. Suramin also significantly enhanced the apoptotic effect of paclitaxel in tumors. In conclusion, suramin improved the activity of paclitaxel in both chemotherapy-naïve and paclitaxel-pretreated animals, without enhancing host toxicity ($\leq 10\%$ body weight loss in all groups). These data have led to the initiation of phase I/II trials of paclitaxel and low-dose suramin combination in advanced metastatic breast cancer patients.

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INTRODUCTION

We recently reported an epigenetic, broad-spectrum mechanism of anticancer drug resistance caused by acidic and basic fibroblast growth factors (aFGF and bFGF) that are expressed in solid tumors. These two proteins at clinically relevant concentrations induce an up to 10-fold resistance to drugs with diverse structures and action mechanisms. The resistance was not due to alteration in drug accumulation (1).

Suramin, a polysulfonyl-naphthylurea, inhibits the binding of a number of polypeptide growth factors such as platelet-derived growth factor, aFGF, bFGF, vascular endothelial growth factor, transforming growth factor- β and insulin-like growth factor-1 to their receptors (2–8). Our earlier studies showed that nontoxic concentrations of suramin (15 $\mu\text{mol/L}$) were sufficient to completely reverse the FGF-induced resistance to paclitaxel, doxorubicin and 5-fluorouracil in cultured prostate tumor cells. We further showed that suramin at nontoxic and subtherapeutic suramin doses significantly enhanced the therapeutic efficacy of chemotherapy, without enhancing the host toxicity, in multiple xenograft tumors in mice, including (a) paclitaxel and doxorubicin in subcutaneous or lung metastases of human prostate PC3 tumors, (b) CPT11 in human colon HT29 xenografts, (c) gemcitabine and paclitaxel in human pancreatic Hs766T xenografts, and (d) mitomycin C in human bladder RT4 xenografts (1, 9–13). Finally, suramin at concentrations below 50 $\mu\text{mol/L}$ also enhanced the activity of 5-fluorouracil in histocultures of human renal cell cancer (14). These encouraging preclinical results have led to phase I/II trials using low-dose suramin to enhance the antitumor activity of paclitaxel plus carboplatin, a standard combination therapy in advanced non-small-cell lung cancer patients, and the results suggest therapeutic benefits (15, 16). Together, the preclinical and clinical results that we have obtained thus far support a beneficial effect of low-dose suramin on the antitumor activity of paclitaxel, which is an important and commonly used drug for breast cancer. This, in turn, has generated interest in using low-dose suramin to treat advanced metastatic breast cancer patients.

It is noteworthy that multiple reports in the literature indicate contradicting effects of bFGF on chemosensitivity. On one hand, similar to our findings in human prostate PC3 and rat prostate MAT-LyLu tumor cells, the addition of exogenous bFGF or overexpression of bFGF confers resistance to etoposide in two small-cell lung cancer cell lines (17), resistance to cisplatin in human bladder tumor cells (18), and resistance to fludarabine in human chronic lymphocytic leukemia cells (19). bFGF also causes resistance of endothelial cells to radiation, fibroblasts to *N*-(phosphonacetyl)-L-aspartic acid, muscle cells to sublethal ischemic insult, and neural cells to neomycin analog G418, both *in vitro* and *in vivo* (20–23). The chemoresistance in small-cell lung cancer cells is mediated through activation of the mitogen-activated protein kinase pathway resulting in *bcl-2*

up-regulation. On the other hand, several reports have indicated that the addition of exogenous bFGF or overexpression of bFGF enhances the sensitivity of human breast MCF7 tumor cells to multiple chemotherapeutic agents (*e.g.*, cisplatin, etoposide, 5-fluorouracil, doxorubicin, carboplatin, and docetaxel; refs. 24–27). The mechanism of chemosensitization is presumably due to *bcl-2* down-regulation and *bax* up-regulation. bFGF also causes mitogenesis and G₁ arrest in breast cancer cell lines, with the final net effect on proliferation depending on the cell lines, passages, and culture condition (24, 28). The bFGF-mediated chemosensitization was also found in other cell lines, including human prostate PC12, NIH3T3 fibroblasts, and two ovarian and one pancreatic tumor cell lines. In general, chemosensitization by bFGF is not due to growth inhibition and requires pretreatment for at least 4 hours at levels that are much higher than required for its more well-known mitogenic effect (5–10 *versus* 0.5 ng/mL; ref. 27).

There are also conflicting reports on the role of bFGF in the response of breast cancer patients to chemotherapy. Higher expression of bFGF is associated with improved overall and disease-free survival (29), whereas lower bFGF levels (<400 pg/mg) in primary breast tumors were significantly correlated with increased tumor size and higher tumor stage (30). On the other hand, one study showed no significant relationship between tumor bFGF levels and survival (31), and two studies showed that enhanced bFGF expression is associated with aggressive disease and worse prognosis in primary, nodal-negative disease (32, 33).

The opposing observations on the bFGF effects on chemosensitivity in breast cancer cells raise the question whether suramin will result in chemosensitization or, through FGF inhibition, abrogate the chemosensitization effect of bFGF and thereby lower the sensitivity of breast cancer to chemotherapy. This question needs to be addressed before the initiation of clinical studies. The goal of the present study was, therefore, to evaluate the effect of low-dose suramin on the activity of paclitaxel in immunodeficient mice bearing MCF7 xenograft tumors. Two studies were conducted. The first one evaluated the drug effects in chemotherapy-naïve MCF7 xenograft tumors. Furthermore, to determine whether previous chemotherapy affects the benefits offered by suramin, we also studied the effect of low-dose suramin in animals previously treated with paclitaxel.

The present study used several pharmacodynamic end points to evaluate drug effects, some of which are less conventional but may be more clinically relevant. Specifically, we used the standard methodologies in preclinical *in vivo* antitumor activity evaluation including monitoring the changes in tumor size over time and median survival time (MST). In addition, because our goal is to translate the preclinical data to clinical application, we believe it necessary to use comparable definitions of activity under preclinical and clinical settings. For example, in a preclinical study, a drug demonstrating tumor growth delay compared with the control group is interpreted as an active compound. In contrast, in patients, continued tumor growth (even with a growth delay) would be considered progressive disease, and the test drug would be considered inactive; and only drugs that cause tumor regression would qualify as active drugs.

Hence, we incorporated the end points used in clinical oncology such as response rate (extent and onset) and time to tumor progression (TTP) in our data analysis. To further quantify the benefits offered by suramin, we also measured the tumor growth/regression rate, and, in the case of the paclitaxel-treated group, the onset and rate of tumor regrowth.

MATERIALS AND METHODS

Chemicals and Reagents. Paclitaxel was obtained from Hande Tech (Houston, TX), suramin from Sigma (St. Louis, MO), cefotaxime sodium from Hoechst-Roussel (Somerville, NJ), other cell culture supplies from Life Technologies, Inc. Laboratories (Grand Island, NY).

Cell and Tumor Cultures. Human breast MCF7 tumor cells were a gift of Dr. Kenneth Cowan (National Cancer Institute, Bethesda, MD). Tumor cells were maintained as monolayer cultures at 37°C in a humidified atmosphere containing 5% CO₂ in RPMI 1640 culture medium supplemented with 9% fetal bovine serum, 2 mmol/L L-glutamine, 90 µg/mL gentamicin, and 90 µg/mL cefotaxime.

Animal and Drug Treatment Protocols. Female athymic nude mice (5–6 weeks old) were purchased from the National Cancer Institute (Bethesda, MD). Mice were housed in air-filtered laminar flow cabinets and cared for under aseptic procedures in accordance with the institutional guidelines. Estradiol pellets (SE-121, Innovative Research of America, Sarasota, FL) that released estradiol over 60 days were implanted subcutaneously 1 day before tumor implantation and again on day 61.

Stock solutions of paclitaxel were prepared by dissolving the drug in a vehicle solution (EtOH:cremophor, 50:50 v/v) at a concentration of 15 mg/mL. Stock solutions of suramin were prepared by dissolving the drug in physiologic saline at a concentration of 1.1 mg/mL. Vehicle or paclitaxel stock solution were mixed with physiologic saline or 1.1 mg/mL suramin (10:90 v/v). A dosing solution (200 µL) was intravenously injected, over 1 minute via a tail vein, twice weekly for 3 weeks. The suramin dose was 10 mg/kg, and the paclitaxel dose was 15 mg/kg. A separate pharmacokinetic study in normal mice (*i.e.*, without tumors) indicated that the selected suramin dose yielded a peak plasma concentration of 50 µmol/L immediately after the bolus dose administration and a concentration of 1 µmol/L at 72 h; this suramin dose and concentration range was previously found sufficient to reverse the FGF-induced chemoresistance but had no *in vivo* antitumor activity against other xenograft tumors (1, 9). The selected paclitaxel dose yielded a 5-ng/mL concentration at 72 hours,³ which was near its IC₅₀ in MCF7 monolayer cultures (34).

Two studies were conducted. The first one evaluated the drug effects in chemotherapy-naïve MCF7 xenograft tumors. Furthermore, to determine whether previous chemotherapy affects the benefits offered by suramin, we also studied the effect of low-dose suramin on paclitaxel activity in animals that were

³ Unpublished data.

previously treated with paclitaxel and in which the residual tumors had regrown.

For the study in chemotherapy-naïve animals, MCF7 cells were harvested from subconfluent cultures with trypsin and were injected subcutaneously into the back flanks of a mouse on both sides ($2 \cdot 10^6$ cells/site in 200 μ L of physiologic saline). Drug treatment was started at 2–3 weeks after tumor implantation, when the tumor reached a size of at least 5 mm.

As shown in Results, six paclitaxel treatments were sufficient to eradicate the MCF7 tumors in some chemotherapy-naïve mice. To study the effect of low-dose suramin on paclitaxel activity in paclitaxel-pretreated mice, a group of mice received only four paclitaxel treatments (without suramin). After tumor regrew to ~ 1 cm in length, mice were treated with physiologic saline, or paclitaxel (15 mg/kg) with or without suramin (10 mg/kg), twice weekly for 3 weeks.

In all studies, animals were randomized to the control and treatment groups according to their initial tumor weight, such that all groups within each study had comparable initial tumor sizes. The second randomization criterion was the initial body weight.

***In vivo* Chemosensitivity Evaluation.** Drug treatment effects were measured with several pharmacodynamic end points. The first set of end points was based on the commonly used tumor size measurement. The widths and lengths of tumors were measured with a caliper. Tumor volume was calculated as $(\text{width})^2 \times (\text{length})/2$. Tumor size was measured twice weekly for 1 month and then once weekly until 120–136 days after the start of treatment. Animals were euthanized when the tumor volume exceeded 1 cm³ or on day 136. As discussed in Introduction, we also used criteria similar to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria to analyze the tumor size data (35). A mouse that showed 50% reduction in the tumor volume, sustained in two consecutive measurements, was considered a partial response (PR). A mouse that showed no evidence of tumor in two consecutive measurements (≥ 1 week) was considered a complete response (CR). A mouse that showed new tumor lesions or 50% increase in the total tumor volume relative to the initial total tumor volume was considered progressive disease (PD). A mouse that did not satisfy the criteria for PR or PD was considered stable disease (SD).

The effect of chemotherapy on tumor growth rate was measured by two methods. The first was the growth rate during treatments; tumor-regrowth rate was calculated as (difference of initial and final tumor volume since the initiation of treatment) divided by (number of days over which the measurements were made). The second method was to measure the tumor regrowth after termination of chemotherapy. In the chemotherapy-naïve group, some animals, after drug treatments, had residual tumors that resumed growth; the onset and rate of the tumor regrowth were measured. Note that tumor regrowth data were obtained only for the chemotherapy-naïve group and not for the paclitaxel-pretreated group. This was because the latter group had relatively large tumors that were close to the allowed limit of 1 cm³ (volume) by institutional guidelines, and we were not able to continue the tumor regrowth measurement. In this case, tumor regrowth rate was measured as (difference of smallest and largest tumor volume during the regrowth phase) divided by

(number of days over which the tumor size measurements were made).

The second set of pharmacodynamic end points was survival, including progression-free survival, and overall survival. This was conducted in the chemotherapy-naïve animals, and data analysis was accomplished with standard Kaplan-Meier plots. Because the tumors of the paclitaxel-pretreated animals were excised for morphologic evaluation, they were not studied for survival.

The third set of pharmacodynamic end points was tumor morphologic changes induced by drug treatments. These included the extent of apoptosis induction and the reduction of residual nonapoptotic fraction, as described previously (1). Briefly, 3 days after the last drug treatment, a mouse was anesthetized, and its tumors were excised, fixed in 10% formalin, and embedded in paraffin. Five- μ m histologic sections were obtained and stained with hematoxylin and eosin. The number of tumor cells, apoptotic cells, and nonapoptotic cells in randomly selected microscopic fields at $\times 400$ were determined. Apoptotic cells were identified by their condensed nuclei and membrane blebbing. Others and we have shown that apoptotic cells identified by these morphologic changes are identical to the apoptotic cells identified by the terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) method (36, 37). On average, we counted 10 fields per animal, or >930 , >570 , and >430 cells in the control, single-agent paclitaxel, and the paclitaxel/suramin combination groups, respectively. This study was conducted in the paclitaxel-pretreated group and was not possible in the chemotherapy-naïve group because of the small size of the residual tumors.

Statistical Analysis. Statistical significance of the differences in nonapoptotic cell density, apoptotic fraction, and body weight changes were assessed by the Tukey test after ANOVA. Statistical analysis for differences in tumor growth rate was determined by ANOVA for repeated measures. We used the log-rank test to analyze the differences in survival fractions, Fisher exact test for overall response rate (*i.e.*, PR, CR, SD plus PD), and unpaired Student's *t* test for onset and duration of responses as well as tumor growth and regrowth rates.

RESULTS

Effect of Low-Dose Suramin on Paclitaxel Activity in Chemotherapy-Naïve Tumors *In vivo*. The control and the three treatment groups had similar initial tumor weights and body weights (Table 1). The results on the pharmacodynamic end points; *i.e.*, change in tumor size, response rate, TTP, survival time, tumor regrowth, apoptotic index, and fraction of residual nonapoptotic cells, were as follows.

Figure 1 shows the tumor growth rates in the saline-treated controls and drug-treated groups and the effects of drug treatments. The tumor volume in the control group reached about 400% of the initial size on day 21 or 4 days after the last treatment. The single-agent suramin group showed similar tumor growth curves as the control group. For the single-agent paclitaxel group, the tumor size increased during the first week, but tumor regression was evident by day 10 and continued throughout the remaining treatments such that the tumor volume was reduced by $\sim 60\%$ on day 21 ($P < 0.05$ compared with

Table 1 Enhancement of paclitaxel activity by low-dose suramin.

	Chemotherapy-naïve mice				P	Paclitaxel-pretreated mice		
	Control (n = 5)	Suramin (n = 5)	Paclitaxel (n = 17)	Combination (n = 19)		Paclitaxel (n = 6)	Combination (n = 6)	P
Body weight, g								
Pretreatment	22.8 ± 1.71	22.3 ± 0.93	21.8 ± 2.17	22.7 ± 2.17	0.32	24.2 ± 3.11	22.0 ± 2.29	0.19
Posttreatment	22.8 ± 2.00	21.8 ± 1.22	21.7 ± 2.78	21.6 ± 3.52	0.91	21.9 ± 4.29	21.5 ± 3.66	0.84
Initial tumor volume, mm ³	463 ± 235	471 ± 187	260 ± 176	317 ± 131	0.35	838 ± 465	900 ± 459	0.83
Total response rate, %	0	0	70.6	100	0.02*	0	50	0.09*
PR	0	0	47.1	36.8	0.02*	0	50	0.09*
CR	0	0	23.5	63.2	0.02*	0	0	0.09*
SD	0	0	17.6	0	0.02*	66.7	50	0.09*
PD	100	100	11.8	0	0.02*	33.3	0	0.09*
Median TTP, day	3	3	73	136	<0.01	10	>21	<0.01
MST, day	21	21	73	>136	<0.01	NM	NM	NA
Tumor growth rate during treatment, % per day	0.13 ± 0.10	0.13 ± 0.08	-0.03 ± 0.01	-0.04 ± 0.03	0.02	0.01 ± 0.03	-0.02 ± 0.02	0.05
Tumor regrowth rate after treatment, % per day	NA	NA	0.05 ± 0.06	0.01 ± 0.02	0.01	NA	NA	NA

NOTE. MCF7 tumor-bearing mice were treated with saline, suramin (10 mg/kg), paclitaxel (15 mg/kg), or paclitaxel/suramin combination. The tumor growth rates during treatments were calculated as the difference of initial and final tumor volume divided by the number of days. The tumor regrowth after termination of chemotherapy in the chemotherapy-naïve group was calculated as the difference of largest and smallest tumor volume during the regrowth phase divided by the number of days over which the tumor size measurements were made. + and - indicate increase and decrease in tumor size (*i.e.*, growth and regression), respectively. The criteria for response are described in Materials and Methods. Data are mean ± SD. *P* values for the differences between single-agent paclitaxel and combination groups are shown.

Abbreviations: PR, partial response; CR, complete response; SD, stable disease; PD, progressive disease; TTP, time to tumor progression; MST, median survival time; NM, not measured because animals were euthanized to harvest tumors for morphological evaluation; NA, not applicable.

* Differences in the overall response between single-agent paclitaxel and combination groups (Fisher's exact test).

control and single-agent suramin group). In the combination group, tumor regression started immediately after the first treatment and continued throughout the entire 21-day treatment period. The extent of tumor regression in the combination group was also significantly greater compared with the single-agent paclitaxel group (77 *versus* 60% for tumor volume; *P* < 0.05).

Table 1 shows the response rates and times to achieve responses. Consistent with the tumor growth characteristics, no response was observed for the control and single-agent suramin groups. The single-agent paclitaxel group showed a total response rate of 70.6%, and the combination group showed a higher rate of 100%. Other major differences between the two groups are the 2.6-fold higher CR rate, more rapid tumor regression, and shorter time to attain PR (35 ± 33 days *versus* 84 ± 53 days) and CR (34 ± 26 days *versus* 72 ± 45 days).

Figure 1B shows the Kaplan-Meier plot of the progression-free survival data. All of the animals in the control and single-agent suramin groups showed progression during treatments; the median TTP was the same at 3 days. In contrast, none of the animals treated with paclitaxel, with or without suramin, showed progression while receiving drug treatments. Single-agent paclitaxel significantly prolonged the median TTP to 73 days, which was further improved to 136 days by the addition of suramin.

Figure 1C shows the Kaplan-Meier plot of the overall survival data. The MST for the control and single-agent suramin groups were identical at 21 days. Single-agent paclitaxel significantly prolonged MST to 73 days (*P* < 0.001, compared with the control group). The addition of suramin further prolonged the survival; MST was longer than 136 days, or the last day of the experiment (*P* < 0.01).

In the animals in the single-agent paclitaxel and the paclitaxel/suramin combination groups that had residual tumors after the drug treatments ended, tumor growth resumed after a lag time (Fig. 1A). For the single-agent paclitaxel group, tumor regrowth occurred on day 4 to 49 days after the termination of drug treatment (mean ± SD, 23.4 ± 15.4 days; median, 21 days). In comparison, the combination group showed a significantly later onset of tumor regrowth, beginning on day 14 to 105 days after termination of drug treatment (mean ± SD, 52.9 ± 29.8 days; median, 42 days; *P* < 0.01). The rate of tumor regrowth rate in the combination group was also significantly lower, at ~20% of the regrowth rate in the single-agent paclitaxel group.

Effect of Low-Dose Suramin on Paclitaxel Activity in Paclitaxel-Pretreated Tumors *In vivo*. As indicated above, six treatments with single-agent paclitaxel eradicated tumors in 24% of animals. Hence, for the study of drug effect in paclitaxel-pretreated tumors, which required residual tumors, we used only four paclitaxel treatments given over 2 weeks. The tumor regression and responses during paclitaxel treatments in this group were similar to the data over the same duration in the chemotherapy-naïve group after receiving four treatments (data not shown). With the four-treatment regimen, 75% (12 of 16) animals showed residual tumors that regrew to ~1 cm in length in 10 to 63 days after treatment termination. These animals were retreated with single-agent paclitaxel or paclitaxel/suramin combination, twice weekly for three weeks. Fig. 2A shows the changes in tumor volume over time. The tumor size in the control group increased with time. Retreatment with single-agent paclitaxel caused tumor growth delay but did not produce tumor regression; the best response was 67% SD, and the

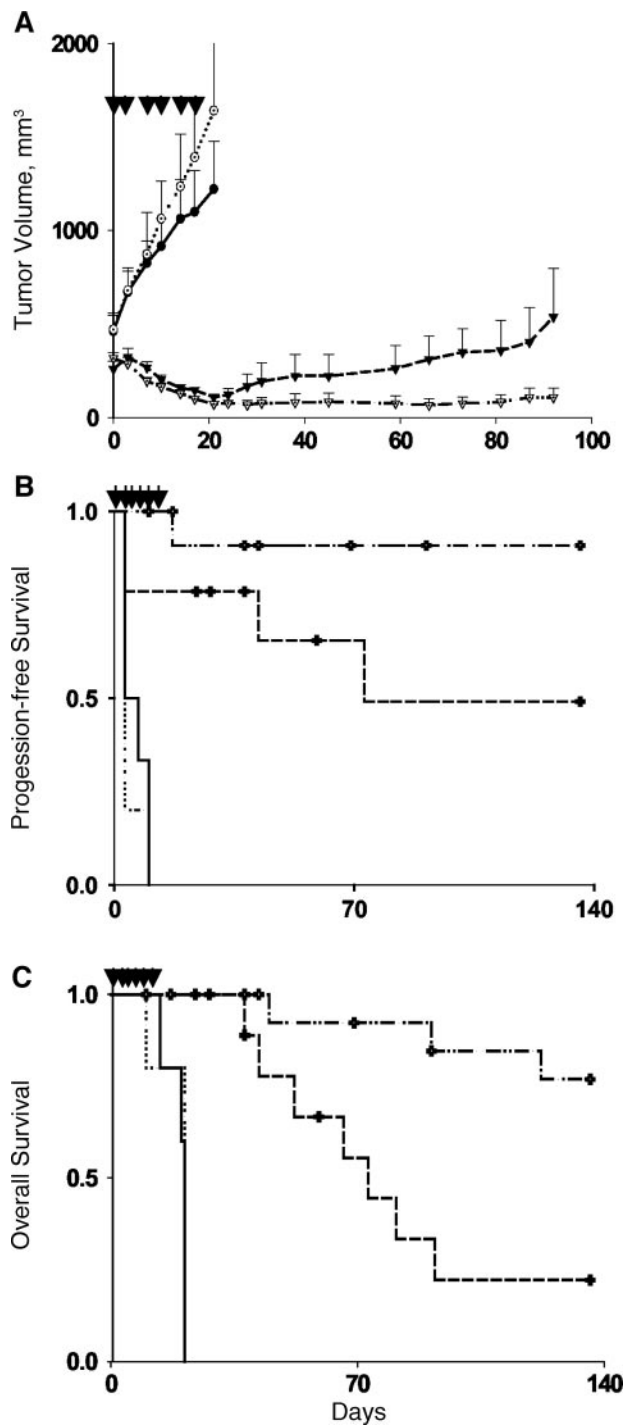


Fig. 1 Effect of low-dose suramin on antitumor activity of paclitaxel in chemotherapy-naïve mice bearing MCF7 xenograft tumors. Animals with well-established, subcutaneously implanted MCF7 tumors were treated with physiologic saline (●, control, $n = 5$), 10 mg/kg suramin (○, $n = 5$), 15 mg/kg paclitaxel (▼, $n = 17$), or paclitaxel/suramin combination (▽, $n = 19$). **A**, changes in tumor volume. Mean and one SEM. **Arrows**, times of treatments. **B**, Kaplan-Meier plots of progression-free survival. **C**, Kaplan-Meier plots of overall survival. *From left to right*, control, single-agent suramin, single-agent paclitaxel, and paclitaxel/suramin combination. Accidental deaths (*i.e.*, unrelated to tumors) are censored and represented by +.

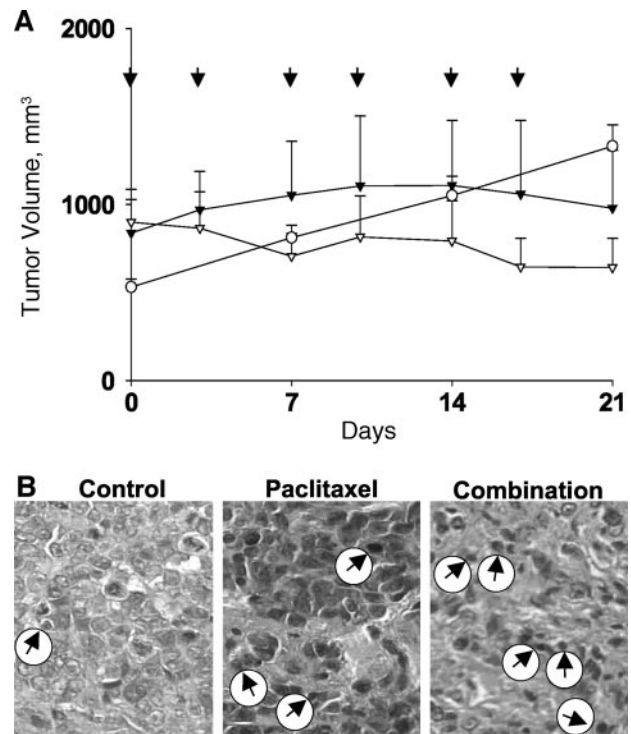


Fig. 2 Effect of low-dose suramin on antitumor activity of paclitaxel in paclitaxel-pretreated mice bearing MCF7 xenograft tumors. Mice bearing well-established, subcutaneously implanted MCF7 tumors were treated with 15 mg/kg paclitaxel twice weekly for 2 weeks. After the initial tumor regression, tumors were allowed to regrow to ~10 mm in length. The mice were then retreated with physiologic saline (○, control, $n = 6$), 15 mg/kg paclitaxel (▼, $n = 6$), or paclitaxel plus 10 mg/kg suramin (▽, $n = 6$), twice weekly for 3 weeks. **A**, changes in tumor volume. Mean + one SEM. **B**, tumor morphology. *Arrows*, apoptotic cells. The greater apoptotic index and the fewer nonapoptotic cells are notable in the combination group compared with the control and single-agent paclitaxel groups (data summarized in Table 2).

median TTP was 10 days, which occurred on the day of the fourth treatment. The paclitaxel/suramin combination was able to completely inhibit tumor growth and cause tumor regression, resulting in 50% PR and 50% SD as the best responses observed on day 21 or 4 days after the sixth and last treatment.

Differences in the Responses of Chemotherapy-Naïve and Paclitaxel-Pretreated Groups to Single-Agent Paclitaxel and Paclitaxel/Suramin Combination.

Comparisons of the antitumor activities of various treatments in the chemotherapy-naïve and paclitaxel-pretreated groups indicate substantial differences. In general, the pretreated group was less sensitive to paclitaxel, with or without suramin coadministration. For single-agent paclitaxel, the chemotherapy-naïve group but not the pretreated group showed tumor regression and major responses. Conversely, a small fraction of animals in the naïve group showed PD during the course of treatment, compared with the pretreated group (12 versus 33%). The TTP was also significantly shorter in the pretreated group (73 versus 10 days). Likewise, the combination group showed 63% CR in the naïve group but failed to produce major responses in the pretreated group, and the extent of tumor regression was attenuated in the

Table 2 Tumor morphological data

	Viable cell number (n/field)			Apoptotic cell number (n/field)			Apoptotic cell fraction, %		
	Average	Range	Median	Average	Range	Median	Average	Range	Median
Control	223 ± 12	210–237	222	11 ± 2	8–13	11	5 ± 1	3–6	4
Paclitaxel	97 ± 44*	67–167	71	59 ± 20†	32–86	59	40 ± 16*	16–55	46
Combination	39 ± 40*	0–96	35	75 ± 19†	46–98	73	73 ± 24*	44–99	71

NOTE. Mice that were previously treated with paclitaxel were retreated with paclitaxel (15 mg/kg), with or without coadministration of suramin (10 mg/kg), twice weekly for 3 weeks. Tumors were excised 3 days after treatments ended and were processed for morphological evaluation. Data are expressed as mean ± SD. We did not study single-agent suramin because this treatment in the paclitaxel-pretreated animals would result in tumor size (1-cm³ volume) that exceeded the upper limit according to institutional guidelines. Data for the control group was obtained using chemotherapy-naïve animals (*i.e.*, animals shown in Fig. 2A).

* $P < 0.05$ Tukey after ANOVA compared with all other groups.

† $P < 0.05$ Tukey after ANOVA compared with control.

pretreated group (11 versus 44%; $P = 0.003$). The combination also produced less tumor regression in the pretreated group compared with single-agent paclitaxel in the naïve group (11 versus 23%; $P = 0.049$). It was noted, however, that the pretreated groups, because of tumor regrowth, showed a 3-fold larger tumor size compared with the naïve groups.

Effect of Drug Treatments on Animal Body Weight.

Table 1 summarizes the body weight changes during drug treatments. All of the groups showed body weight loss of ≤10%, and no differences were observed between groups ($P = 0.91$), which indicated no significant toxicity due to paclitaxel, and that low-dose suramin did not enhance the paclitaxel toxicity.

Effect of Drug Treatments on Tumor Morphology.

This determination was conducted on the paclitaxel-pretreated animals and not the chemotherapy-naïve animals because of their small residual tumors after drug treatments. Figure 2B shows the tumor morphology and Table 2 summarizes the results. Tumors in the control group showed 5% apoptosis and 95% nonapoptotic cells. Paclitaxel significantly enhanced the apoptotic cell fraction to 40% and, accordingly, decreased the nonapoptotic cell fraction. The addition of low-dose suramin further significantly increased the apoptotic fraction to 73%.

This study did not have the single-agent suramin group because this treatment in the paclitaxel-pretreated animals would result in tumor size (1 cm³ volume) that exceeded the upper limit according to institutional guidelines. However, other studies in our laboratory have shown that low-dose suramin does not produce appreciable apoptosis *in vivo* (1, 9, 10).

DISCUSSION

The present study was motivated by the finding that low-dose suramin reversed the FGF-induced resistance to paclitaxel in prostate tumors implanted in mice and by the phase II data in non-small-cell lung cancer patients, which suggested therapeutic benefits by adding low-dose suramin to the paclitaxel/carboplatin combination therapy (1). Our goal was to determine whether low-dose suramin enhances the activity of paclitaxel in breast cancer patients. The major conclusions are as follows. First, subcutaneously implanted, chemotherapy-naïve MCF7 xenograft tumors are highly sensitive to paclitaxel; paclitaxel treatments caused significant tumor regression and prolonged TTP and MST. Paclitaxel was able to reduce the growth of

paclitaxel-pretreated tumors but was not able to produce tumor regression. Second, suramin, at nontoxic and subtherapeutic doses, significantly enhanced the activity of paclitaxel in chemotherapy-naïve and paclitaxel-treated MCF7 tumors, including improved responses (*i.e.*, higher rate and longer duration), reduced tumor regrowth after treatment termination (*i.e.*, slower onset and slower growth), and prolonged TTP and MST. Importantly, the paclitaxel/suramin combination was able to stabilize or cause minor tumor regression in the paclitaxel-pretreated group, whereas single-agent paclitaxel failed to do the same. Consistent with these data on the whole animal level, suramin also enhanced the apoptotic effect of paclitaxel on the microscopic level. Third, based on the different activity of single-agent paclitaxel and paclitaxel/suramin combination in the chemotherapy-naïve and paclitaxel-pretreated groups, we hypothesize (a) that the resistance mechanism developed after paclitaxel treatment is different from the mechanism in chemotherapy-naïve animals and cannot be completely reversed by suramin, and (b) that greater benefits will result from the suramin chemosensitization in chemotherapy-naïve animals. However, that the lower activity of chemotherapy in the pretreated group may be due to the larger initial tumor size cannot be ruled out. Fourth, this study points to an animal model of paclitaxel resistance. This model may be more realistic compared with typical drug resistance models induced first *in vitro* by exposure of tumor cells to high and/or clinically unachievable concentrations of chemotherapeutics and, therefore, may provide additional insights in the development of *in vivo* drug resistance and may enable drug activity evaluation in a second-line setting. To our knowledge, this represents the first *in vivo* paclitaxel chemoresistance model. There are two reports on the development of a cyclophosphamide-resistant acute myeloid leukemia model and a melphalan-resistant ovarian cancer model (38, 39).

Our earlier data in cultured prostate tumor cells, showing that the addition of bFGF antibody did not enhance the synergy between doxorubicin and suramin, suggest a bFGF-mediated mechanism under *in vitro* conditions (1, 9, 10). However, suramin has additional multiple pharmacological effects, including inhibition of other growth factors and other targets such as reverse transcriptase, DNA polymerase, interleukin-2, tumor necrosis factor α , topoisomerase II protein kinase C, RNA polymerase, and transforming growth factor β (40). Additional studies are needed to elucidate the mechanisms of the *in vivo* chemosensitization effect of suramin.

Suramin has shown some activity in prostate cancer and has been tested in a wide variety of solid tumors, either as single agent or in combination with other chemotherapeutics. Suramin has been evaluated clinically since the early 1980s. At least 33 trials have been published (*e.g.*, 41–50). In all of these trials, suramin was used as a cytotoxic agent at therapeutic plasma concentrations of between 100 and 200 $\mu\text{mol/L}$. Suramin has also been tested in breast cancer patients as an antiangiogenic, again requiring the maintenance of concentrations above 140 $\mu\text{mol/L}$ (46). At these concentrations, suramin shows considerable toxicities and only modest activity in patients. Furthermore, suramin-containing combination therapy did not show a benefit over monotherapy. This has led to recommendations by multiple investigators, against its future use (44–50). The major difference between the previous clinical studies with suramin and our ongoing studies is the intended use of suramin and, accordingly, the selection of the dose/concentration. In the present study, suramin is used to reverse bFGF-induced resistance, an effect that requires only a 10-to-20- $\mu\text{mol/L}$ concentration, which has no cytotoxicity in cultured tumor cells nor toxicity in animals or patients. Another important consideration is the concentration-dependent effect of suramin on cell cycle kinetics. Suramin at concentrations above 50 $\mu\text{mol/L}$ arrests cells in the G_1 phase (51–53). A blockage in the G_1 phase may prohibit cells from progressing to the later phases such as the S and M phases, in which other agents exert their action. An example is the combination of suramin and radiation; suramin at 50- $\mu\text{mol/L}$ concentration caused cell cycle arrest in the G_1 phase, which, in turn, resulted in antagonism with radiation, which is most effective in the G_2 -M phase (53). In contrast, the 10-to-20- $\mu\text{mol/L}$ concentration that we used to reverse the FGF-induced resistance does not cause G_1 arrest and, therefore, is not expected to negatively affect the activity of chemotherapeutic agents.

Finally, the present study used, in addition to the conventional tumor size and MST measurements, several pharmacodynamic end points to evaluate treatment activity in tumor-bearing mice. These included response rate, TTP, and tumor growth rate. Although these end points are less conventional in preclinical studies, they are used routinely in clinical drug evaluation. On the basis of this consideration and the fact that these end points can be readily obtained from the tumor size data, we propose to incorporate these end points in data analysis, to reduce the differences in the drug evaluation criteria under preclinical and clinical setting. This, in turn, may enable differentiating candidate drugs that cause only tumor growth delay from the candidates that cause tumor regression and, as discussed in the Introduction, may close the gap in the definition of active drugs under preclinical and clinical settings.

In summary, results of the present study indicate that low-dose suramin enhanced the activity of paclitaxel in chemotherapy-naïve and paclitaxel-pretreated MCF7 xenograft tumors in mice, without enhancing host toxicity. The data further suggest that the addition of suramin produces greater benefits in chemotherapy-naïve subjects, with respect to significant and clinically meaningful tumor regression. These results have led to the initiation in our institution of a phase I/II trial of using low-dose suramin in combination with paclitaxel in advanced, metastatic, chemotherapy-naïve, and taxane-pretreated breast cancer patients.

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