Nontoxic Doses of Suramin Enhance Activity of Doxorubicin in Prostate Tumors

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ABSTRACT

We recently reported that acidic and basic fibroblast growth factors (aFGF and bFGF) confer a broad-spectrum chemoresistance in solid tumors, and that inhibitors of these proteins enhanced the antitumor activity of several anticancer drugs. The present study investigated the effect of FGF inhibitors on doxorubicin activity in human prostate PC3 tumors. In vitro studies, conditioned medium (CM) obtained from histocultures of rat MAT-LyLu lung metastases and different combinations of recombinant FGF induced a 7- to 15-fold doxorubicin resistance. Suramin had no effect on the doxorubicin activity in the absence of CM or FGF, but reversed the CM- and FGF-induced resistance by >90% at concentrations that had no cytotoxicity (i.e., 1–17 μM suramin). In the in vivo study, immunodeficient mice bearing well established, subcutaneous PC3 tumors (~100 mg in size) were treated intravenously with doxorubicin (5 mg/kg) and suramin (10 mg/kg), administered twice weekly for 3 weeks. The suramin dose, selected to yield plasma concentration of below 50 μM, had neither antitumor activity nor toxicity. Doxorubicin alone reduced tumor growth rate by ~60%, reduced the density of nonapoptotic tumor cells by ~60%, enhanced the apoptotic cell fraction by 4-fold, and reduced the body weight by ~15% (p < 0.05 compared with control). Addition of suramin to doxorubicin therapy did not increase weight loss but significantly enhanced the antitumor effect, resulting in complete inhibition of tumor growth, an additional 3-fold reduction in the density of nonapoptotic tumor cells, and an additional 2-fold enhancement of the apoptotic tumor cell fraction (p < 0.05 compared with all other groups). These data indicate significant enhancement of the effectiveness of doxorubicin in prostate tumors by nontoxic and subtherapeutic doses of suramin.

Prostate cancer is the most common malignancy in human males. Hormone refractory prostate cancer has a poor prognosis with an overall median survival of 9 to 18 months (Gudziak and Smith, 1994; Oesterling et al., 1997). Secondary hormonal manipulation may provide symptomatic relief in about 38% of patients and a 40 to 90% subjective response in 30% of patients, with a limited response duration ranging from 3 to 16 months (Daniel et al., 1990; Matzkin and Soloway, 1992). A similar response rate has been achieved with systemic chemotherapy (Perez et al., 1989). None of the presently available treatments produce survival advantage (Gudziak and Smith, 1994; Oesterling et al., 1997). The low response rate and short-lived response in patients underscore the need of developing more effective treatments.

Doxorubicin has been used to treat prostate cancer. Doxorubicin produces one of the highest combined partial and complete objective response rates for single agents, of about 30% (Perez et al., 1989). Using histocultures of human prostate tumors obtained from patients with locally confined and early stage disease, we showed that doxorubicin can produce complete antiproliferation and cell kill. But these effects require doxorubicin concentrations that exceed the clinically achievable plasma concentrations by 25- to 100-fold (Chen et al., 1998). Hence, one approach to improve the efficacy of doxorubicin therapy is to enhance tumor sensitivity to the drug.

The microenvironment of the tumor-bearing organ may play an important role in lowering the chemosensitivity of metastatic tumors (Fidler and Hart, 1990; Fidler et al., 1994). For example, murine colon tumor cells implanted subcutaneously or into different visceral organs show differential sensitivity to doxorubicin, with the subcutaneous tumor being sensitive, whereas the tumors at the metastatic sites (i.e., lung and liver) are insensitive. For this tumor, the chemoresistance of metastatic tumors was correlated with the mdr1 P-glycoprotein (Pgp) overexpression; the Pgp expression was transient and culturing of tumor cells as monolayers resulted in reversal of Pgp expression and chemoresistance (Dong et al., 1994). On the other hand, clinical

ABBREVIATIONS: Pgp, mdr1 P-glycoprotein; aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; BrdU, bromodeoxyuridine; r-aFGF, recombinant-acidic fibroblast growth factor; r-bFGF, recombinant-basic fibroblast growth factor; CM, conditioned medium of rat MAT-LyLu lung metastatic tumors; ANOVA, analysis of variance.
studies show that inhibition of the drug efflux proteins, including Pgp, does not significantly improve the effectiveness of chemotherapy in patients (Broxterman et al., 1996; Ferry et al., 1996), suggesting the existence of other chemoresistance mechanisms.

Using the transplantable, metastatic rat prostate MAT-LyLu tumor, we have shown that the antitumor activity of paclitaxel in lymph node metastases was 20-fold lower than in subcutaneously implanted primary tumors. When the metastatic tumor was reimplanted at the subcutaneous site, the resistance was lost in the second-generation primary tumor but regained in the second-generation metastases (Yen et al., 1996). We subsequently showed that the chemoresistance in lung metastases is caused by acidic and basic fibroblast growth factors (aFGF and bFGF) expressed in solid tumors. These two proteins at clinically relevant concentrations induce an up to 10-fold resistance to the antiproliferative and apoptotic effects of drugs with diverse structures and action mechanisms. The resistance was not due to alteration in drug accumulation (Song et al., 2000). The mechanisms by which aFGF/bFGF induce resistance are unknown. One possibility is alteration of apoptosis; bFGF treatment in neurons and endothelial cells resulted in increased Bel-2 level and reduced Bax level (Karsan et al., 1997; Liu and Zhu, 1999).

We have shown that inhibitors of aFGF and bFGF, including the respective monoclonal antibodies and suramin, completely reverse the FGF-induced resistance (Song et al., 2000). Suramin has multiple pharmacological actions and inhibits multiple growth factors, including aFGF and bFGF (Garrett et al., 1984; Betsholtz et al., 1986; Coffey et al., 1987; Pollak and Richard, 1990). The suramin concentration required to completely reverse the FGF-induced resistance to paclitaxel, doxorubicin, and 5-fluorouracil was 15 μM. This suramin concentration does not cause cytotoxicity in cultured human tumor cells. We further found that suramin, at a dose that delivers a peak plasma concentration of 50 μM, significantly enhances the therapeutic efficacy of doxorubicin against lung tumors in immunodeficient mice, resulting in shrinkage and eradication of well established tumors in animals. The enhanced therapeutic efficacy due to suramin was achieved without enhancing the host toxicity (Song et al., 2000).

The goal of the present study was to evaluate whether suramin enhances the activity of doxorubicin in prostate tumors. In vitro and in vivo studies were performed using human prostate PC3 tumors.

**Materials and Methods**

**Chemicals and Reagents.** Doxorubicin was obtained from Pharmacia Upjohn Inc. (Kalamazoo, MI) or Hoechst-Roussel Inc. (Somerville, NJ), suramin from Sigma (St. Louis, MO), cefotaxime sodium from Hoechst-Roussel Inc., cell culture supplies from Invitrogen (Carlsbad, CA), and bromodeoxyuridine (BrdU) enzyme-linked immunosorbent assay and human recombinant r-aFGF and r-bFGF from Roche Molecular Biochemicals (Indianapolis, IN).

**Cell and Tumor Cultures.** The rat MAT-LyLu tumor was maintained as monolayers or histocultures. Implantation of the MAT-LyLu cells or tumor fragments in the hind limbs of male Copenhagen rats resulted in primary tumors at the implantation site and metastases in lungs. The lung metastases were cultured as histocultures, which were used to collect the tumor-conditioned medium (CM), in a ratio of 50 ml of CM per ~100 mg of tumors. CM collected by this method contains about 0.3 ng/ml aFGF and 0.9 ng/ml bFGF (Song et al., 2000).

Human prostate cancer PC3 cells were purchased from American Type Culture Collection (Rockville, MD). Histocultures and monolayer cultures of rat tumors and monolayers of human PC3 cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂ in RPMI 1640 supplemented with 9% fetal bovine serum, 2 mM L-glutamine, 90 μg/ml gentamicin, and 90 μg/ml cefotaxime.

**In Vitro Drug Activity Evaluation.** Prior to drug treatment, cells were incubated for 4 days with tumor CM, r-bFGF (50 ng/ml), or a combination of r-aFGF plus r-bFGF (0.3 ng/ml plus 1 ng/ml or 1 ng/ml plus 3 ng/ml), as described previously (Song et al., 2000). The medium was renewed every other day. Drug-induced cytotoxicity was measured as inhibition of BrdU incorporation by using enzyme-linked immunosorbent assay. The relationship between drug concentration and effect was analyzed for the 50% inhibitory concentrations (IC50) by computer fitting the concentration-response curves with nonlinear least-squares regression (NLIN; SAS, Cary, NC), as previously described (Au et al., 1998).

**Reversal of CM- and FGF-Induced Resistance by Suramin.** We evaluated the reversal of CM- and FGF-induced resistance to doxorubicin by suramin. Suramin inhibits the action of several polypeptide growth factors, including platelet-derived growth factor, aFGF, bFGF, vascular endothelial growth factor, transforming growth factor-β, and insulin-like growth factor-1 (Garrett et al., 1984; Betsholtz et al., 1986; Coffey et al., 1987; Pollak and Richard, 1990).

The nature of interaction between doxorubicin and suramin was evaluated using two methods. The first method used fixed concentrations of suramin together with increasing concentrations of doxorubicin, i.e., the fixed concentration method. The advantage of this method is that it yields the conventional sigmoidal concentration-effect curves showing the effect of increasing suramin concentration. The second method used fixed ratios of doxorubicin and suramin, i.e., the fixed ratio method. The advantage of the fixed ratio method is that it enables the measurement of the nature of the interaction between doxorubicin and suramin at much broader concentration ranges compared with the fixed concentration method. An additional advantage is that it allows the identification of the optimal concentration ratios of the two drugs that will give the maximal synergy.

For the fixed concentration method, the suramin concentrations were kept constant at 15 μM, while the doxorubicin concentrations were varied between 1 and 1000 nM. For the fixed ratio method, the ratios between doxorubicin concentration and suramin concentration in each combination were kept constant. Cells were treated with solutions containing the two drugs at 0.001 to 100% of their respective initial concentrations. The initial doxorubicin concentration was kept constant at 6 μM, whereas the initial suramin concentrations were varied by 80- to 100-fold.

**Analysis of in Vitro Synergy Data.** The nature of the interaction between doxorubicin and suramin, in the presence of CM or FGF proteins, was analyzed by the combination index method (Chou and Talalay, 1984). The combination index was calculated using eq. 1:

\[
\text{Combination Index} = \frac{IC_{\text{Dox, Comb}}}{IC_{\text{Dox}}} + \frac{IC_{\text{Sur, Comb}}}{IC_{\text{Sur}}} \tag{1}
\]

where IC_{Dox} and IC_{Sur} are the concentrations of doxorubicin and suramin needed to produce a given level of cytotoxicity when used alone, and IC_{Dox, Comb} and IC_{Sur, Comb} are the concentrations needed to produce the same effect when used in combination. A combination index value of 1 indicates additive interaction, values less than 1 indicate synergistic interaction, and values greater than 1 indicate antagonistic interaction. As shown under Results, the synergy between doxorubicin and suramin was due to the reversal of the CM- or
FGF-induced resistance to doxorubicin by suramin. The extent of reversal of drug resistance was calculated using eq. 2:

\[
\text{Extent of Reversal} = \frac{IC_{\text{Dox}, \text{CM}} - IC_{\text{Dox}, \text{CM, Suramin}}}{IC_{\text{Dox}, \text{CM}} - IC_{\text{Dox}}} \tag{2}
\]

where \(IC_{\text{Dox}, \text{CM}}\) is \(IC_{50}\) of doxorubicin in presence of CM or r-bFGF, \(IC_{\text{Dox}, \text{CM, Suramin}}\) is the \(IC_{50}\) of doxorubicin in presence of CM plus suramin or r-bFGF plus suramin, and \(IC_{\text{Dox}}\) is the \(IC_{50}\) of doxorubicin without CM, bFGF, or suramin.

**In Vivo Drug Activity Evaluation: Animal and Drug Treatment Protocols.** Male BALBc/nu.nu mice (5–6-week old) were purchased from the National Cancer Institute (Bethesda, MD). Mice were housed in air-filtered laminar flow cabinets and cared for in accordance with institutional guidelines. PC3 cells were harvested from subconfluent cultures by using trypsin and injected subcutaneously into the flank on both sides of a mouse \((3 \times 10^6\) cells/200 \(\mu\)l of physiological saline in each site).

Stock solutions of doxorubicin and suramin were prepared by dissolving the drug in physiological saline at concentrations of 1 and 2 mg/ml, respectively. Drug treatment was started at 2 weeks after tumor implantation, or when the tumor reached a size of \(-100\) mg.

Mice received intravenous injections, over 1 min via a tail vein, of 200 \(\mu\)l of either physiological saline or a saline solution delivering 5 mg/kg doxorubicin, 10 mg/kg suramin, or a combination of both drugs, twice weekly for 3 weeks (i.e., on days 1, 4, 8, 11, 15, and 18).

The doxorubicin dose was selected based on the dose used in humans (i.e., a 2.4 mg/kg dose in mice was converted to a 28.8 mg/kg dose in mice based on the surface area equivalence method). A separate pharmacokinetic study in normal mice (i.e., without tumors) indicated that the selected suramin dose yielded a peak plasma concentration of 50 \(\mu\)M immediately after the bolus dose administration and a concentration of 1 \(\mu\)M at 72 h (unpublished data). As shown under Results, these suramin concentrations were sufficient to reverse the FGF-induced chemoresistance.

**Tumor Size Measurement.** Because the subcutaneous PC3 tumors were not uniformly spherical, we were not able to obtain accurate measurement of the tumor size by using the conventional width and length measurements. The following procedures were developed. First, a mold of the tumor was obtained using Jeltrate (Dentsply International Inc., Milford, MA). Jeltrate is a polymer used to form dental molds. Briefly, Jeltrate (7 g) was mixed with 19 ml of distilled water, and the mixture was poured into a mold to yield a counter-mold. Three counter-molds were obtained for each tumor. The weights of the three counter-molds were measured and the average weight was used as the tumor weight. Relative tumor growth rate was calculated as percentage of the tumor weight immediately prior to drug treatment. The accuracy of this molding method was investigated by comparing the tumor weight obtained using the molding method with the actual weight of the tumor excised from the animals on the same day; the results showed a positive correlation \((r = 0.90, n = 52)\) and thus confirmed the accuracy of the tumor weight measured by the molding method.

**Histological Evaluation of Tumors.** Four days after completion of drug treatments (i.e., 22 days after the first treatment), animals were euthanized. Tumors were excised, weighed, and fixed in 10% phosphate-buffered neutral formalin and embedded in paraffin. Five micrometer histological sections were prepared and stained with hematoxylin and eosin. The number of tumor cells and the fraction of apoptotic cells in each tumor were determined microscopically. Cells that showed condensed nuclei and blebbing were considered apoptotic; we and others have shown that apoptotic cells identified by these morphological changes are identical to the apoptotic cells identified by the terminal deoxynucleotidyl transferase dUTP nick-end labeling method (Gold et al., 1994; Gan et al., 1996).

Because apoptotic cells disappear over time, a second measure of the extent of apoptosis was the density of nonapoptotic cells in the residual tumors. This was determined by counting the number of nonapoptotic tumor cells in five randomly selected microscopic fields at 400× magnification. On average, we counted 950 ± 260 (mean ± SD) cells/tumor in the control and suramin groups and 540 ± 160 cells/tumor in the doxorubicin group. In the case of combination therapy where only a few tumor-containing fields could be found per tumor, we counted all residual cells (290 ± 100 cells, between 130–565 cells/tumor).

**Statistical Analysis.** Statistical significance for the tumor growth rate was assessed by ANOVA for repeated measures. Statistical significance for other parameters was assessed by ANOVA with Tukey’s test.

**Results**

**Reversal of CM- and bFGF-Induced Doxorubicin Resistance by Suramin.** CM, combinations of low or high concentrations of r-aFGF plus r-bFGF (0.3 plus 1 ng/ml or 1 plus 3 ng/ml), and r-bFGF (50 ng/ml) induced a 6-, 8-, 14-, and 16-fold doxorubicin resistance, respectively, in PC3 cells (Tables 1 and 2). To evaluate the effect of suramin, we used CM and/or r-bFGF to induce resistance and used the fixed concentration method and the fixed ratio method to evaluate the nature of interaction between doxorubicin and suramin. Figure 1 shows the results of the fixed concentration study. Figure 2 shows the results of the fixed ratio study. Tables 1 and 2 summarize the IC\(_{50}\) values and the reversal of the CM- and/or r-bFGF-induced doxorubicin resistance by suramin.

**TABLE 1**

Reversal of CM- and FGF-induced resistance by suramin: results of the fixed concentration method

<table>
<thead>
<tr>
<th>Condition</th>
<th>Doxorubicin IC(_{50})</th>
<th>Resistance Reversal</th>
<th>Combination Index</th>
<th>Synergy, Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>No FGF</td>
<td>8.4 ± 1.1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>+ suramin 15 (\mu)M, no FGF</td>
<td>9.1 ± 1.7</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>+ Low FGF concentrations</td>
<td>67 ± 9.0</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>+ High FGF concentrations</td>
<td>18 ± 17</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>+ Low FGF + suramin 15 (\mu)M(^a)</td>
<td>8.4 ± 1.0(^{bc})</td>
<td>0.30 ± 0.02</td>
<td>3.39 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>+ High FGF + suramin 15 (\mu)M(^a)</td>
<td>8.0 ± 1.0(^{bc})</td>
<td>0.24 ± 0.02</td>
<td>4.20 ± 0.28</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) IC\(_{50}\) of suramin in PC3 cells, in the absence of CM or FGF was 93 ± 19 \(\mu\)M (Fig. 1).

\(^b\) \(p < 0.05\) compared with the group treated with FGF but without suramin.

\(^c\) Not significantly different from the control group with no FGF.
As single agent, suramin had no cytotoxicity at 1 or 17 μM (Fig. 1). In the absence of CM or r-bFGF, suramin had no effect on the doxorubicin activity, as indicated by the nearly identical IC\textsubscript{50} values of doxorubicin in the absence or presence of suramin. In the presence of CM or r-bFGF, addition of suramin reversed the CM- or bFGF-induced resistance. Although the extent of reversal increased with the suramin concentration, relatively low suramin concentrations were required to reverse the CM-induced resistance. For example, 95% of the CM-induced resistance was reversed for combinations with a doxorubicin-to-suramin concentration ratio of 1:120 (i.e., initial concentrations of 6 μM doxorubicin and 720 μM suramin); the corresponding IC\textsubscript{50} values were 10 nM for doxorubicin and 1 μM for suramin. Analysis of the CM results by the combination index method confirms the synergistic interaction between doxorubicin and suramin; the

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**TABLE 2**

Reversal of CM- and r-bFGF-induced resistance by suramin: results of the fixed ratio method

<table>
<thead>
<tr>
<th>Condition (Initial Doxorubicin: Suramin Concentrations)</th>
<th>Doxorubicin Concentration at 50% Effect</th>
<th>Suramin Concentration at 50% Effect</th>
<th>Resistance Reversal</th>
<th>Combination Index</th>
<th>Synergy, fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM-induced resistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CM, no suramin</td>
<td>8.4 ± 1.1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>+ suramin, no CM</td>
<td>9.1 ± 1.7</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>+ CM, no suramin</td>
<td>54 ± 2.7</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>+ CM (6:90)</td>
<td>48 ± 4.8\textsuperscript{a}</td>
<td>0.7 ± 0.1</td>
<td>24 ± 8</td>
<td>0.91 ± 0.24</td>
<td>1.15 ± 0.26</td>
</tr>
<tr>
<td>+ CM (6:180)</td>
<td>26 ± 1.2\textsuperscript{b}</td>
<td>0.8 ± 0.03</td>
<td>59 ± 11</td>
<td>0.51 ± 0.10</td>
<td>2.06 ± 0.37</td>
</tr>
<tr>
<td>+ CM (6:360)</td>
<td>16 ± 0.9\textsuperscript{c}</td>
<td>0.9 ± 0.05</td>
<td>83 ± 5</td>
<td>0.30 ± 0.06</td>
<td>3.39 ± 0.56</td>
</tr>
<tr>
<td>+ CM (6:720)</td>
<td>10 ± 0.8\textsuperscript{d}</td>
<td>1.2 ± 0.1</td>
<td>95 ± 1</td>
<td>0.16 ± 0.03</td>
<td>5.97 ± 1.01</td>
</tr>
<tr>
<td>+ CM (6:3,600)</td>
<td>10 ± 0.7\textsuperscript{e}</td>
<td>5.5 ± 0.4</td>
<td>97 ± 1</td>
<td>0.17 ± 0.02</td>
<td>5.80 ± 0.66</td>
</tr>
<tr>
<td>+ CM (6:7,200)</td>
<td>9.8 ± 1.0\textsuperscript{f}</td>
<td>11 ± 1.1</td>
<td>97 ± 1</td>
<td>0.22 ± 0.03</td>
<td>4.89 ± 0.72</td>
</tr>
<tr>
<td>r-bFGF-induced resistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No r-bFGF</td>
<td>8.9 ± 0.1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>+ suramin, no CM</td>
<td>9.1 ± 1.7</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>+ r-bFGF, no suramin</td>
<td>56 ± 3</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>+ r-bFGF (6:180)</td>
<td>48 ± 2\textsuperscript{b}</td>
<td>1.4 ± 0.1</td>
<td>15 ± 2</td>
<td>0.88 ± 0.02</td>
<td>1.14 ± 0.03</td>
</tr>
<tr>
<td>+ r-bFGF (6:360)</td>
<td>45 ± 3\textsuperscript{b}</td>
<td>2.6 ± 0.2</td>
<td>22 ± 2</td>
<td>0.91 ± 0.10</td>
<td>1.13 ± 0.19</td>
</tr>
<tr>
<td>+ r-bFGF (6:720)</td>
<td>42 ± 4\textsuperscript{b}</td>
<td>4.8 ± 0.5</td>
<td>29 ± 6</td>
<td>0.77 ± 0.06</td>
<td>1.30 ± 0.08</td>
</tr>
<tr>
<td>+ r-bFGF (6:3,600)</td>
<td>23 ± 1\textsuperscript{b}</td>
<td>13 ± 0.6</td>
<td>70 ± 3</td>
<td>0.45 ± 0.03</td>
<td>2.24 ± 0.15</td>
</tr>
<tr>
<td>+ r-bFGF (6:7,200)</td>
<td>14 ± 1\textsuperscript{b}</td>
<td>17 ± 1.2</td>
<td>88 ± 2</td>
<td>0.31 ± 0.01</td>
<td>3.25 ± 0.13</td>
</tr>
<tr>
<td>+ r-bFGF (6:18,000)</td>
<td>9.2 ± 0.9\textsuperscript{c}</td>
<td>26 ± 2.6</td>
<td>100 ± 2</td>
<td>0.24 ± 0.02</td>
<td>4.21 ± 0.30</td>
</tr>
</tbody>
</table>

N.A., not applicable.

\textsuperscript{a} The IC\textsubscript{50} of suramin alone is 93 ± 1.9 μM in the absence of CM or r-bFGF, and 324 ± 16.6 and 366 ± 15.1 μM in the presence of CM and r-bFGF, respectively.

\textsuperscript{b} p < 0.05 compared with the group treated with CM or r-bFGF but without suramin.

\textsuperscript{c} Not significantly different from the control group with no CM nor suramin.

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Fig. 1. Reversal of FGF-induced resistance to doxorubicin by suramin in vitro: results of the fixed concentration method. Drug effect was measured as inhibition of BrdU incorporation. Left, effect of suramin as single agent. Suramin alone without CM or FGF: left solid line (○). Suramin with CM (▼). Suramin with 50 ng/ml bFGF (◇). Right, effect of doxorubicin and reversal of resistance by suramin. Combinations of r-aFGF and r-bFGF were used to induce resistance in PC3 tumor cells. The suramin concentrations were kept constant at 15 μM, whereas doxorubicin concentrations were varied from 0.1 to 1000 nM. Control without FGF or suramin: left solid line (○). Without FGF but with suramin (▼, overlaps with control curve). Combinations of r-aFGF plus r-bFGF, at low (0.3 plus 1 ng/ml of respective proteins, ▷) and high protein concentrations (1 plus 3 ng/ml, □), without suramin. Combinations of r-aFGF/r-bFGF at low (◻) and high (●) protein concentrations, with 15 μM suramin (both curves overlap with the control curve). Mean and 1 S.D. Some S.D. values are smaller than the symbols.
maximal synergy of about 6-fold was also achieved for combinations that contained relatively low initial suramin concentrations (i.e., \( \leq 720 \mu M \)). Qualitatively similar findings were obtained when r-bFGF was used to induce resistance. The resistance induced by CM and r-bFGF (50 ng/ml) was identical at 6-fold. A comparison between the CM and r-bFGF results indicated two differences, as follows. 1) The reversal of r-bFGF-induced resistance required higher suramin concentrations. Ninety percent reversal was achieved at a doxorubicin-to-suramin concentration ratio of 1:1200 (i.e., initial concentrations of 6 \( M \) doxorubicin and 7200 \( M \) suramin); the corresponding IC\(_{50}\) was 14 nM for doxorubicin and 17 \( M \) for suramin. 2) The extent of synergy between doxorubicin and suramin was lower when r-bFGF was used to induce resistance (i.e., \( \sim 3 \)- versus \( \sim 6 \)-fold for CM-induced resistance).

**Enhancement of In Vivo Doxorubicin Activity by Suramin.** Figure 3 shows the tumor growth in the saline-treated controls and the three groups treated with single agents or with the combination of doxorubicin and suramin. The four groups showed similar initial tumor weights and initial body weight (Table 3). At the end of the 22-day experiment, the tumor size in the control group increased by about 3.5-fold. At the selected dose, suramin alone had no antitumor effect or toxicity, which is consistent with the previous results in other mouse tumor models (Chahinian et al., 1998; Song et al., 2000). Doxorubicin alone reduced the tumor growth by about 60%, reduced the density of nonapoptotic cells by \( \sim 60\% \), increased the fraction of apoptotic cells in the residual tumors by \( \sim 4\)-fold, and reduced the body weight by \( \sim 15\% \). Addition of suramin to doxorubicin therapy did not enhance weight loss but significantly enhanced the antitumor effect, resulting in complete inhibition of tumor growth, an additional 3-fold reduction in the density of nonapoptotic tumor cells, and an additional 2-fold enhancement of the apoptotic tumor cell fraction. It is noted that the increase in apoptotic cell fraction and the reduction of the density of nonapoptotic cells did not result in a parallel decrease in tumor size. This is because the space formerly occupied by tumor cells persisted in the tumors (Fig. 3).

**Discussion**

Results of the present study indicate that suramin, at nontoxic concentrations and doses, significantly enhanced the antitumor activity of doxorubicin in cultured human prostate tumor cells, and in mice bearing subcutaneous human prostate xenograft tumors. The findings of substantial synergy between doxorubicin and suramin at nontoxic suramin concentrations support the use of low and nontoxic suramin dose/concentration to enhance the antitumor activity of doxorubicin.

Our results further showed a different requirement of suramin for reversing the resistance induced by CM and by 50 ng/ml r-bFGF and a different extent of synergy between doxorubicin and suramin under these two conditions, even though both conditions induced the same extent of resistance (i.e., 6-fold). We previously showed that the CM contains 0.3 ng/ml aFGF and 0.9 ng/ml bFGF, and that the CM-induced resistance is due in part to aFGF and in part to bFGF. Hence, the difference in the potency of suramin in reversing the resistance induced by CM and 50 ng/ml r-bFGF is probably due to the difference in the suramin-mediated inhibition of aFGF, bFGF, and/or r-bFGF.

Our overall goal is to develop a new approach to treat prostate cancer. We elected to use suramin to reverse the FGF-induced resistance in part because its clinical pharmacological data are readily available. The following discussion outlines the current status on the clinical development of suramin and the differences between the previous approach and our current approach.

Suramin has shown some activity in prostate cancer (Ahmann et al., 1991; Reyno et al., 1995; Small et al., 2000); the therapeutic plasma concentration is between 100 to 200 \( M \) (140–280 \( \mu g/ml \)) (Reyno et al., 1995). The two important limitations of suramin are 1) its broad spectrum of toxicity, including neurotoxicity, renal toxicity, adrenal insufficiency, and immune- and glycosaminoglycans anticoagulant-mediated blood dyscrasias (Horne et al., 1988; La Rocca et al., 1990; Ahmann et al., 1991; Figg et al., 1994; Kobayashi et al., 1996); and 2) difficulty in dose administration due to its
Fig. 3. Enhancement of in vivo antitumor activity of doxorubicin by suramin. Top, reduction of tumor size by drug treatment. Changes in tumor size were expressed as percentage of initial tumor size. Animals with well-established, subcutaneously implanted PC3 tumors were treated with physiological saline (control, ○), 10 mg/kg suramin (●), 5 mg/kg doxorubicin (□), and doxorubicin plus suramin (■). Mean and 1 S.D. *p < 0.01 compared with all other groups by ANOVA for repeated measures. Bottom, histological sections (400×).

TABLE 3
Enhancement of in vivo antitumor effect of doxorubicin by suramin

Human prostate PC3 tumor cells were injected subcutaneously into the right and left flank regions of immunodeficient mice. After 2 weeks or when tumors reached a size of about 100 mg, animals received an intravenous injection of physiological saline, 5 mg/kg doxorubicin, 10 mg/kg suramin, or a combination of both drugs. The average pretreatment weights for four groups ranged from 20 to 22 g. Each animal had two tumors. For each tumor, cell density and apoptotic cell fraction were determined using five randomly selected microscopic fields at 400× magnification. Mean ± S.D.

<table>
<thead>
<tr>
<th>Treatment (No. of Animals)</th>
<th>Initial Tumor Size (No. of Tumors)</th>
<th>End-of-Experiment Tumor Size (No. of Tumors)</th>
<th>Density of Nonapoptotic Cells per 400× Field (No. of Fields)</th>
<th>Apoptotic Cell Fraction (No. of Fields)</th>
<th>End-of-Experiment Body Weight (% of Pretreatment Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6)</td>
<td>0.094 ± 0.029 (12)</td>
<td>0.331 ± 0.113 (12)</td>
<td>186 ± 60 (60)</td>
<td>9 ± 6 (60)</td>
<td>105 ± 4</td>
</tr>
<tr>
<td>Suramin (6)</td>
<td>0.084 ± 0.036 (12)</td>
<td>0.280 ± 0.085 (12)</td>
<td>164 ± 52 (60)</td>
<td>11 ± 8 (60)</td>
<td>108 ± 5</td>
</tr>
<tr>
<td>Doxorubicin (7)</td>
<td>0.103 ± 0.032 (14)</td>
<td>0.207 ± 0.089 (14)</td>
<td>69 ± 32 (70)*</td>
<td>38 ± 13 (70)*</td>
<td>83 ± 5*</td>
</tr>
<tr>
<td>Doxorubicin + suramin (7)</td>
<td>0.098 ± 0.023 (14)</td>
<td>0.114 ± 0.072 (14)*</td>
<td>26 ± 17 (67)*</td>
<td>61 ± 16 (67)*</td>
<td>85 ± 6*</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with all other groups.

#p < 0.05 compared with control group.
exceedingly long terminal plasma half-life of over 21 days (Dorr and Von Hoff, 1994; Jodrell et al., 1994). Bayesian pharmacokinetics have been used to individualize the suramin treatment schedule. A typical treatment consists of a test dose, a loading dose on day 1, plus 15 to 20 doses over 70 to 80 days to maintain a steady-state plasma concentration of 100 to 200 μM. By using this complex dosing schedule, a randomized phase III trial in prostate cancer patients shows moderate palliative benefit, slight increase in time to tumor progression, and a greater proportion of patients with >50% decline in prostate-specific antigen (Small et al., 2000). The relatively modest activity of suramin led to the development of combination therapies of suramin with other agents, where suramin was again given at doses that result in 100 to 200 μM concentration; these combinations have either shown limited benefit or have resulted in toxicity that discouraged further evaluation of these regimens (Rapport et al., 1993; Falcone et al., 1998; Tu et al., 1998).

The major difference between the previous clinical studies with suramin and our ongoing study is the intended use of suramin and, accordingly, the selection of the dose/concentration. In previous studies, suramin was used as a therapeutic agent and therefore required the maintenance of a target concentration of 100 to 200 μM. In the current study, suramin is used to reverse the FGF-induced resistance, an effect requiring ≤20 μM, which has minimal or no cytotoxicity in cultured tumor cells nor toxicity in animals or patients. Another important consideration is the concentration-dependence of effect on suramin cell cycle kinetics. Suramin at concentrations above 50 to 100 μM arrests cells in the G_1 phase (Qiao et al., 1994; Howard et al., 1996; Palayoor et al., 1997). A blockage in the G_1 phase may prohibit cells from progressing to the later phases such as the S and M phases where other agents exert their action. An example is the combination of suramin and radiation; suramin at 50 μM concentration caused cell cycle arrest in the G_1 phase that in turn resulted in antagonism with radiation, which is most effective in the G_2/M phase (Palayoor et al., 1997). In contrast, the 10 to 50 μM concentration that we used to reverse the CM- or FGF-induced resistance does not cause G_1 arrest and therefore is not expected to negatively affect the activity of the chemotherapeutic agent.

In summary, results of the present study indicate that low and nontoxic doses of suramin significantly enhance the in vitro and in vivo antitumor activity of doxorubicin, and support a new treatment paradigm with combinations of chemotherapy with aFGF/bFGF inhibitors to treat prostate cancer. In addition to doxorubicin, other candidate chemotherapeutics include antimicrotubules such as docetaxel (Taxotere), which have shown significant activity against prostate cancer (Picus and Schultz, 1999). We further found that suramin enhanced the activity of paclitaxel in human xenograft lung metastases. The latter finding has led to a phase I/II trial of suramin, paclitaxel, and carboplatin in nonsmall-cell lung cancer patients in our institution.

References

Qiao L, Pizolo J, and Meltamed M (1984) Effects of suramin on expression of

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