Antitumor Activity. Part 2: Efficacy Evaluations

Michael C. Alley, Melinda G. Hollingshead, Christine M. Pacula-Cox, William R. Waud, John A. Hartley, Philip W. Howard, Stephen J. Gregson, David E. Thurston, and Edward A. Sausville

ABSTRACT

Pyrrolo[2,1-c][1,4]benzodiazepine dimer SJG-136 (NSC 694501) selectively cross-links guanine residues located on opposite strands of DNA, and exhibits potent in vitro cytotoxicity. In addition, SJG-136 is highly active in vivo in hollow fiber assays. In the current investigation, SJG-136 was evaluated for in vivo efficacy in 10 tumor models selected on the basis of sensitivity of cells grown in the hollow fiber and in vitro time course assays: LOX IMVI and UACC-62 (melanomas); OVCA-3 and OVCA-5 (ovarian carcinomas); MDA-MB-435 (breast carcinoma); SF-295 and C-6 (gliomas); LS-174T (colon carcinoma); HL-60 TB (promyelocytic leukemia); and NCI-H522 (lung carcinoma). SJG-136 was active against small (150 mg) and large (250–400 mg) xenografts with tumor mass reductions in all 10 models. In addition, significant growth delays occurred in nine models, cell kill in six models ranged between 1.9 and 7.2 logs, and there were 1 to 4/6 tumor-free responses in six models. SJG-136 occurred in nine models, cell kill in six models ranged between 1.9 and 7.2 logs, and there were 1 to 4/6 tumor-free responses in six models. SJG-136 is highly active in vivo in hollow fiber assays. In the current investigation, SJG-136 was evaluated for in vivo efficacy in 10 tumor models selected on the basis of sensitivity of cells grown in the hollow fiber and in vitro time course assays: LOX IMVI and UACC-62 (melanomas); OVCA-3 and OVCA-5 (ovarian carcinomas); MDA-MB-435 (breast carcinoma); SF-295 and C-6 (gliomas); LS-174T (colon carcinoma); HL-60 TB (promyelocytic leukemia); and NCI-H522 (lung carcinoma). SJG-136 was active against small (150 mg) and large (250–400 mg) xenografts with tumor mass reductions in all 10 models. In addition, significant growth delays occurred in nine models, cell kill in six models ranged between 1.9 and 7.2 logs, and there were 1 to 4/6 tumor-free responses in six models. SJG-136 is active following i.v. bolus injections, as well as by 5-day continuous infusions. Of all of the schedules tested, bolus administrations for 5 consecutive days (q x 5) conferred the greatest efficacy. SJG-136 is active over a wide dosage range in athymic mouse xenografts: on a q x 5 schedule, the maximum-tolerated dose was ~120 mg/kg/dose (total dose: 0.6 mg/kg = 1.8 mg/m²) and the minimum effective dose in the most sensitive model (SF-295) was ~16 mg/kg/dose (total dose: 0.08 mg/kg = 0.24 mg/m²). Results of this study extend the initial in vivo observations reported in the reference above and confirm the importance of expediting more detailed preclinical evaluations on this novel agent in support of phase I clinical trials in the United Kingdom and the United States, which are planned to commence shortly.

INTRODUCTION

SJG-136 (NSC 694501) was reported by Thurston et al. (e.g., refs. 1–4) to be one of a series of rationally designed pyrrolobenzodiazepine dimers which bind covalently in the minor groove of DNA as described in the preceding manuscript by Hartley et al. (5).

SJG-136 was tested in National Cancer Institute’s (NCI’s) in vitro 60 cell line screen and found to be very potent and to exhibit a multilong differential pattern of activity (6) in which some cell types were markedly more sensitive over several doses of drug concentration. These findings coupled with initial evidence of in vivo antitumor activity in the hollow fiber assay prompted more detailed efficacy evaluations of SJG-136 in human tumor xenografts using several potential treatment regimens. The preliminary efficacy evaluations previously reported (7) have been expanded to include additional xenograft models and treatment schedules as well as a comparison of the in vivo efficacy of SJG-136 with that of bizelesin in the present article.

MATERIALS AND METHODS

Materials

The pyrrolo[2,1-c][1,4]benzodiazepine dimer, SJG-136 (NSC 694501), initially was synthesized (3, 4) and supplied by David E. Thurston and colleagues of the Cancer Research UK Gene Targeted Drug Design Research Group, School of Pharmacy, University of the United States NCI. These materials were inventoried by the Drug Synthesis and Chemistry Branch of the Developmental Therapeutics Program, NCI as samples 1 and 2 (absolute purity values unknown but >85%). Subsequent experimental testing required the synthesis of additional batches of material by Starks Associates, Inc., of Buffalo, NY (samples 3, 4, and 5). Analyses of the latter samples revealed somewhat differing levels in absolute purity: sample 3, 86.3%; sample 4, 89.4%; and sample 5, 97.6%. Stock solutions were prepared with 100% analytical grade ethanol and then partitioned into replicate vials, dried under nitrogen, and stored at ~70°C. For drug testing, SJG-136 was first solubilized in 100% ethanol or 100% DMSO and then diluted to final vehicle concentrations of 1% ethanol/0.9% NaCl/0.05% Tween 80, 1% DMSO/0.9% NaCl/0.05% Tween 80, or 50% DMSO/0.5% propylene glycol for efficacy testing (unless otherwise noted) or to 0.25% ethanol/RPMI 1640 culture medium/20% fetal bovine serum for in vitro time course assays.

Bizelesin (U-77779, NSC 615291) was synthesized by the Upjohn Company (Kalamazoo, MI) and provided to NCI. Sample 12 was prepared for efficacy testing in 2% N,N-dimethylacetamide (Sigma Chemical Corp., St. Louis, MO), 10% Enulphor EL-620P (GAF Corp., Wayne, NJ) and 85% (v/v) normal NaCl. Sample 99 was a clinical formulation of bizelesin supplied in 2-mL sterile vials each containing 5 µg bizelesin/mL of special diluted vehicle. The diluent consisted of 100 µL of PET (polyethylene glycol 400, ethanol, Tween 80, 6:3:1, v/v), 1 mg of citric acid, and 0.9% NaCl in quantity sufficient to achieve 1.00 mL. Drug solutions were prepared by dilution in 0.9% NaCl just before injections.

In vitro Time Course Assay

Methods for cell culture and characterization, drug preparations, and conventional in vitro drug sensitivity testing have been described previously (8). The cell lines and concentration ranges of experimental agents to be evaluated in the more specialized concentration × time (c × t) assays were chosen on the basis of 60 cell line screening data and from the results of hollow fiber assays. As described elsewhere (9, 10), exposure to an experimental agent for increasing periods of time, followed by drug removal, permits quantitation of drug activity conferred by each of several exposure durations ranging from ~1 to 144 hours. Comparison to platoes not exposed to drug permits determination of concentration and times of exposure conferring 50% net growth inhibition (GI50), total growth inhibition (TGI), and 50% cell kill (LC50). From plots of composite c × t data one can readily determine the minimum exposure conditions (both concentration and time) required to achieve cytostatic and/or cytocidal activity in a given cell line. By comparing the relative sensitivities of multiple cell lines, the most sensitive cell types for in vitro efficacy evaluations in xenograft models can be identified.
Table 1. Efficacy testing in selected human tumor xenografts

<table>
<thead>
<tr>
<th>Tumor model</th>
<th>Treatment schedule (drug sample no.)</th>
<th>Optimum dose (µg/kg/injection)</th>
<th>Percent body weight loss (control)</th>
<th>Minimum percent of control growth</th>
<th>Percent growth delay</th>
<th>Net log cell kill</th>
<th>Tumor free</th>
</tr>
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<td>MDA-MB-435</td>
<td>q4d×3 (2)</td>
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<td>41</td>
<td>1/6</td>
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<td>UACC-62</td>
<td>q4d×3 (2)</td>
<td>450</td>
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<td>28</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>q7d×5 (4)</td>
<td>54</td>
<td>20.2 (9.8)</td>
<td>26</td>
<td>150</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>LOX IMVI†</td>
<td>q5d×5 (4)</td>
<td>122</td>
<td>22.2 (14.4)</td>
<td>11</td>
<td>463</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>OVCAR-3</td>
<td>q4d×3 (3)</td>
<td>450</td>
<td>13.9 (1.5)</td>
<td>10</td>
<td>55</td>
<td>1/8</td>
<td></td>
</tr>
<tr>
<td>OVCAR-5</td>
<td>q4d×3 (3)</td>
<td>270</td>
<td>13.9 (0.6)</td>
<td>9</td>
<td>114</td>
<td>1.9</td>
<td>1/8</td>
</tr>
<tr>
<td>SF-295</td>
<td>q5d×5 (4)</td>
<td>81</td>
<td>10.2 (0.0)</td>
<td>1</td>
<td>227</td>
<td>3.2</td>
<td>1/6</td>
</tr>
<tr>
<td></td>
<td>q7d×5 (4)</td>
<td>120</td>
<td>5.3</td>
<td>2</td>
<td>196</td>
<td>3.2</td>
<td>2/6</td>
</tr>
</tbody>
</table>

* Efficacy testing was performed using early-stage tumor models unless otherwise noted. Samples of SJG-136 (NSC 694501) were all prepared fresh just before injection and administered i.v. in 1% ethanol or 1% DMSO in 0.9% NaCl at a volume of 0.1 mL/10 g body weight. No drug-related deaths occurred with any treatments at optimum doses.
† Body weight losses often accompany i.v. treatment regimens. % body weight loss for vehicle control-treated mice versus nontreated controls are shown in parentheses for treatment groups, which exhibit >10% body weight loss. No drug-related deaths occurred with any treatments at optimum doses.
¶ Net log cell kill at the end of therapy based on the tumor growth delay of the optimal treatment group in comparison with the control group.
§ Fraction of tumor-free mice for the optimum treatment group at termination of each experiment.
†† Efficacy testing was performed using an advanced-stage tumor model.

** Bizelesin (U-77779, NSC 615291) was prepared fresh just before injection and administered i.v. as follows: a vehicle of 2% N,N-dimethylacetamide and 10% Emulphor EL-620P in 0.9% NaCl was given in a volume of 0.1 mL/10 g body weight.
§§ In a vehicle of 100 µL PET (polyethylene glycol 400, ethanol, 0.05% Tween 80, 63:3:1, v/v), and 1 mg of citric acid per mL 0.9% NaCl was given in a volume of 0.1 mL/10 g body weight.

In vivo Efficacy Evaluations

The origin, development, characterization, and use of human tumor xenografts for in vivo efficacy evaluations were described in detail by Stinson et al. (11), Dykes et al. (12), and Plowman et al. (13). In brief, human tumor xenografts were maintained in immunocompromised mice by serial passage of tumor fragments after establishment of tumors from in vitro cell culture samples. The host animals for these tumor models were random-bred, athymic/NCr (nu/nu) mice or athymic rats (NCI Animal Production Program; NCI-Frederick, Frederick, MD). Animals, housed in Microisolator cages (Lab Products, Inc., Maywood, NJ), were fed commercial mouse food and water ad libitum. Tumor fragments (~20 mg) were used to implant mice s.c. with MDA-MB-435, UACC-62, LOX IMVI, OVCAR-3, OVCAR-5, SF-295, HL-60 TB, and NCI-H522 xenograft tumors unless noted otherwise in the data tables. Rats were implanted with C-6 (14) and LS-174 using a suspension of 10⁷ cultured cells.

For drug treatment experiments, one of two protocols was followed: most initial drug efficacy experiments were performed using an early-stage tumor treatment protocol. Experimental compound treatment was generally initiated within 5 to 7 days after tumor implantation or when the tumors achieved a mass of ~150 mg. More detailed efficacy evaluations were performed using advanced-stage tumor models in which treatments generally began ~7 to 14 days after tumor implantation when tumors were 200 to 400 mg in mass. Drug administration schedules used in this study were as follows: single bolus treatment (q7dx3), daily for five treatments (q5dx5), every fourth day for three treatments (q4dx3), every seventh day for two or three treatments (q7dx2, q7dx3), as well as continuous infusions.

For continuous infusion experiments, athymic nude mice prepared with indwelling i.v. jugular catheters were obtained from the Animal Production Facility (NCl-Frederick). The mice were acclimated for several days before s.c. inoculation of human tumor xenografts. Three to 5 days after tumor inoculation the mice were implanted s.c. with the Alzet osmotic pumps (model 2001; Durect Corp., Cupertino, CA), which had been loaded with the test agent 12 to 16 h before implantation. The outflow end of the pump was modified by removal of the silicone covering to expose the outflow tip. The i.v. catheter was flushed with 15 µL of the test agent and then connected directly to the osmotic pump by inserting the pump flow regulator into the end of the catheter. At the end of the 5-day infusions, the pumps were removed, and the ends of the catheters were sealed. For each surgical step, mice were anesthetized with methoxyflurane gas (Medical Developments Australia, Springvale, Victoria, Australia) by inhalation.

Schedules and treatments with SJG-136 or bizelesin were given as described in Tables 1–4. In each experiment, SJG-136 or bizelesin was evaluated at several dose levels (ranging from toxic to nontoxic) with each dosage administered to 6 to 8 mice (10 rats). Tumor-bearing control mice or rats (10 to 20/experiment) were treated with the appropriate vehicle as summarized in the data tables. Host body weights and tumor sizes were measured twice weekly, and the tumor weight (in mg) was calculated using the formula {length (mm) × [width (mm)]²}/2. Antitumor activity was assessed on the basis of the

Table 2. Efficacy testing of SJG-136 (NSC 694501) in nude rat xenograft models

<table>
<thead>
<tr>
<th>Tumor model</th>
<th>Treatment schedule (drug sample no.)</th>
<th>Dose (µg/kg/injection)</th>
<th>Percent body weight loss</th>
<th>Minimum percent of control growth</th>
<th>Percent growth delay</th>
<th>Net log cell kill</th>
<th>Tumor free</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-6</td>
<td>q7d×5 (5)</td>
<td>40</td>
<td>0.8</td>
<td>24</td>
<td>49</td>
<td>1/6</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>27</td>
<td>6.3</td>
<td>47</td>
<td>20</td>
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<td></td>
<td></td>
<td>18</td>
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<td>0.0</td>
<td>41</td>
<td>43</td>
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<td></td>
</tr>
</tbody>
</table>

* Efficacy testing was performed using early-stage tumor models. Samples of SJG-136 (NSC 694501) were prepared fresh just before injection and administered i.v. in 1% ethanol in 0.9% NaCl at a volume of 0.1 mL/10 g body weight. No drug-related deaths occurred with treatment at optimum dosages.
percent of control growth (%C), tumor growth delay, and net log cell kill as follows:

**Minimum Percent of Control Growth (Minimum %C).** The median tumor weight for each treated (T) and control (C) animal group is calculated for each time point at which tumors are measured. To determine the %C, the median tumor weight of each treated group (Medₜ) at each measurement time is divided by the median tumor weight of the control group (Medₜ) at the same time point. For each treatment group, the lowest value of the %C is identified from all of the time points, and this minimum %C is taken as a principle index of efficacy. To correct for the starting tumor weight in advanced-stage tumors, the median tumor weight on the day of first treatment (staging day) is subtracted from the median tumor weight at each subsequent time point before calculating the %C, which are used to calculate a %C as follows: early-stage protocol, %C = (Medₜ/Medₜ) × 100; and advanced-stage protocol, %C = (Medₜ/Medₜ) × 100 when Medₜ > 0 or %C = (Medₜ/Medₜ) × 100 when Medₜ < 0 and Tₚ is the median tumor weight at the start of treatment.

**Tumor Growth Delay.** This is expressed as a percentage by which the treated animal tumor weight is delayed in attaining a specified tumor size compared with controls using the formula: \( \frac{(D_T - D_C)}{D_C} \times 100 \), where \( D_T \) and \( D_C \) are the median times in days for treated and control animals, respectively, to attain the specified size (excluding tumor-free and any drug related deaths). The growth delay is expressed as a percentage of the control to account for the growth rate of the tumor because a growth delay based on \( D_T - D_C \) alone varies in significance with differences in tumor growth rates.

**Net Log Cell Kill.** An estimate of the proportion of log₁₀ units of cells killed at the end of treatment is calculated as: \( \frac{[\text{Tumor weight on day of first treatment}]}{\text{Tumor weight on day of first treatment}} \times 0.301/\text{median doubling time} \), where (\( L_T - L_C \)) is the difference between the median day a specified tumor weight is achieved in the treated (\( L_T \)) and control (\( L_C \)) groups, and the median doubling time is defined as the time required for the tumor to increase from 200 to 400 mg.

### RESULTS

**Assessment of In vitro c × t Activity.** Cell lines found to be sensitive in the 60 cell line in vitro screen and in the hollow fiber assay (5) were selected for evaluations of in vitro c × t activity and in vivo efficacy. The in vitro c × t assay, which allows determination of the drug concentrations and exposure times that produce growth inhibition and/or cell kill, was used to evaluate the activity of SJG-136 (NSC 694501) in a group of 12 leukemia, melanoma, glioma, and carcinoma cell lines. The agent possesses time- and concentration-dependent activity in all 12 tumor cell lines. Typical c × t profiles for 8 cell lines are shown in Fig. 1: a GI₅₀ was achieved with ≥6 hours of exposure to concentrations as low as 0.010 to 0.063 nmol/L, and a persistent lethal effect (LC₅₀) was achieved with ≤1 to 6 hours of exposure to 0.66 to 1.9 nmol/L in highly sensitive cell lines (e.g.,

### Table 3

<table>
<thead>
<tr>
<th>Tumor model</th>
<th>Treatment schedule (drug sample no.)</th>
<th>Dose (µg/kg/injection)</th>
<th>Percent body weight loss (control)</th>
<th>Minimum percent of control growth</th>
<th>Percent growth delay</th>
<th>Net log cell kill</th>
<th>Tumor free</th>
</tr>
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<tbody>
<tr>
<td>SF-295</td>
<td>qd×5 (4)</td>
<td>122</td>
<td>14.2 (1.0)</td>
<td>1</td>
<td>248</td>
<td>3.8</td>
<td>3/6</td>
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<tr>
<td></td>
<td>81</td>
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<td>122</td>
<td>27.5 (17.8)</td>
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<td>783</td>
<td>7.2</td>
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<td>81</td>
<td>18.9</td>
<td>15</td>
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<td>5.1</td>
<td>3/6</td>
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</table>

* Refer to methodology and to Table 1A footnotes for explanation of the efficacy parameters summarized in this table.

† SJG-136 (NSC 694501) was prepared fresh just before injection and administered i.v. in 1% ethanol or 1% DMSO in 0.9% NaCl at a volume of 0.1 mL/10 g body weight. No drug-related deaths occurred with any treatments at optimal dosages.

‡ Efficacy testing was performed using an advanced-stage tumor model.

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### Table 4

<table>
<thead>
<tr>
<th>Tumor model</th>
<th>Treatment schedule (drug sample no.)</th>
<th>Optimum dose† (µg/kg/injection)</th>
<th>Percent body weight loss (control)</th>
<th>Minimum percent of control growth</th>
<th>Percent growth delay</th>
<th>Net log cell kill</th>
<th>Tumor free</th>
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<tr>
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<tr>
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<td>5/6</td>
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<td>13.5 (10.5)</td>
<td>-100</td>
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<td>13.4</td>
<td>6/6</td>
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<td></td>
<td>qd×5 (5)</td>
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<td>13.4 (6.0)</td>
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<td>20.1 (9.3)</td>
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<td>14.0 (11.6)</td>
<td>-100</td>
<td>274</td>
<td>1.0</td>
<td>3/6</td>
</tr>
<tr>
<td></td>
<td>qdt×5 (5)</td>
<td>122**</td>
<td>11.1 (0.0)</td>
<td>-100</td>
<td>356</td>
<td>2.0</td>
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</table>

* Refer to methodology and to Table 1A footnotes for explanation of the efficacy parameters summarized in this table. No drug-related deaths occurred with any treatments at optimal dosage.

† SJG-136 (NSC 694501) was prepared fresh just before injection and administered as follows: # 1% ethanol in 0.9% NaCl given i.v. at a volume of 0.1 mL/10 g body weight/injection.

‡ 50% DMSO/50% polyethylene glycol given i.v. by Alzet pump at a volume of 24 µL/24 hour for 5 days.

§ 100% DMSO given i.v. at a volume of 0.01 mL/10 g BW/injection.

¶ 5% DMSO in 0.9% NaCl given i.v. at a volume of 0.1 mL/10 g body weight/injection.

** 1% DMSO in 0.9% NaCl given i.v. at a volume of 0.1 mL/10 g body weight/injection.

§§ Unevaluable (ue) parameter due to large fraction of long-term tumor regressions and/or tumor-free survivors.

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NCI-H522). In contrast, less sensitive cell lines required ≧12-hour exposures of 0.18 to 1.0 nmol/L to achieve growth inhibition and 1.1 to 13 nmol/L to achieve lethal activity. The tumor cell lines LOX IMVI and SF-295 exhibited $c \times t$ profiles, which were intermediate in sensitivity between the most sensitive cell lines, NCI-H522 and HL-60 TB, and the least sensitive cell lines, MDA-MB-435, UACC-62, and LS-174T (each of which exhibited in vivo sensitivity to this agent).

**Initial In vivo Human Tumor Xenograft Evaluations.** SJG-136 (NSC 694501) was initially evaluated for in vivo efficacy in each of six s.c. human tumor xenograft models in athymic mice and two s.c. tumor models in athymic rats. These include UACC-62 and LOX IMVI (melanoma), OVCAR-3 and OVCAR-5 (ovarian carcinoma), MDA-MB-435 [breast carcinoma or melanoma (15, 16)], SF-295 and C-6 (glioma), and LS-174T (colon carcinoma). In athymic mice, SJG-136 (NSC 694501) was active in each of the six tumor models after i.v. bolus drug delivery with minimum %C values ranging from 0 to 38%. Additionally, significant growth delays occurred in each model with multilog cell kill in four models and 1 to 4/6 tumor-free status in three models. These data are summarized in Table 1A. A qd×5 schedule conferred better efficacy than a q4d×3 schedule in each of the four models tested on both schedules. The purest preparation of SJG-136 (sample 5) was more active based on the minimum %C and growth delay data than were the less pure preparations (samples 3 and 4).
Efficacy Evaluations of Bizelesin. OVCAR-3 and SF-295 xenograft models were used for comparative efficacy testing of bizelesin (NSC 615291) versus SJG-136. Under optimal SJG-136 treatment conditions, OVCAR-3 and SF-295, as well as UACC-62 and LOX IMVI (Table 1A), each exhibited highly responsive indices of antitumor activity. A comparison of the SJG-136 results with those observed for bizelesin under optimal treatment conditions in this study (Table 1B) and with those previously reported for UACC-62 and LOX IMVI (ref. 17; Table 2) indicates that SJG-136 is more active than bizelesin in three of the four xenograft models tested in terms of minimum %C, growth delay, and the frequency of tumor-free animals.

Tumor Xenografts in Athymic Rats. SJG-136 (NSC 694501) treatment was also evaluated in athymic rats to determine whether the agent would confer anticancer activity in an alternate rodent species. SJG-136 was well tolerated and efficacious in athymic rats bearing C-6 (rat glioma) and LS-174 (human colon cancer) tumor grafts. NSC 694501 freshly prepared in the 1% ethanol vehicle just before i.v. bolus injections was active and well tolerated at doses of 27 and 40 \( \mu g/\text{kg/dose} \), \( qd \times 5 \) (total dose: 0.14 to 0.20 mg/kg = 0.81 to 1.2 mg/m\(^2\)) as summarized in Table 2.

Characterization of Effective Dosage Range and Profile of Antitumor Activity. The early-stage SF-295 and advanced-stage LOX IMVI xenograft models were selected for assessing the maximum-tolerated dose and minimum effective dose of SJG-136, as well as to additionally characterize the in vitro time course profile of antitumor activity for this agent. The data (Table 3) indicate that freshly prepared SJG-136 was active over a wide dose range in athymic mice. For a \( qd \times 5 \) schedule, the maximum-tolerated dose was 120 \( \mu g/\text{kg/day} \) (total dose: 0.6 mg/kg = 1.8 mg/m\(^2\)), and the minimum effective dose was 16 \( \mu g/\text{kg/day} \) (total dose: 0.08 mg/kg = 0.24 mg/m\(^2\)). In each model, the three highest doses tested were associated with multilog cell kills and very prominent growth delays exceeding 200%. Even at the lowest doses tested, SJG-136 achieved levels of activity that met one or both criteria for antitumor activity (minimum %C \( \leq \) 40% and/or growth delay > 50%).

Antitumor activity and tumor regrowth profiles in these two xenograft models after single courses of treatment are shown in Figs. 2 and 3. On a \( qd \times 5 \) schedule, the early-stage SF-295 model and the advanced-stage LOX IMVI model each exhibited a delayed pattern of tumor growth suppression or regression. With LOX IMVI, growth suppression did not become fully manifest for at least 4 days after termination of treatment. Moreover, with these regimens, tumor growth suppression or regression were maintained for 12 to 38 days in the LOX model as depicted in Fig. 3 and for 20 to 35 days in the SF-295 model as depicted in Fig. 2.

In vivo Efficacy Evaluations Using Alternative Treatment Schedules. The profiles of antitumor activity observed for the LOX IMVI and SF-295 models (Table 3; Figs. 2 and 3) and the identification of additional sensitive tumor cell lines using in vitro time course assays (Fig. 1) prompted us to assess alternate treatment regimens in the LOX IMVI model and to evaluate the effectiveness of SJG-136 in other human tumor xenograft models. We also evaluated the \( qd \times 1 \) and \( qd \times 5 \) regimens in an advanced-stage HL-60 TB s.c. xenograft and the \( qd \times 1 \), \( qd \times 5 \), and \( q7d \times 3 \) regimens in the advanced-stage NCI-H522 s.c. xenograft. Results are summarized in Table 4. Clearly, SJG-136 is well tolerated and highly active on each schedule and in each of these human tumor xenograft models, not only according to tumor growth delays but even more so in terms of the incidence of tumor-free animals in each of the models. The HL-60 TB was the most sensitive model, with six of six tumor-free animals in two experiments (one studied until 42 days after cessation of treatment). In this series of experiments, the \( qd \times 5 \) schedule confers better antitumor activity than the single dose schedule in each of these models. A \( q7d \times 2 \) schedule in LOX IMVI and a \( q7d \times 3 \) schedule in NCI-H522 are also effective although not optimal.

The early-stage LOX IMVI model treated with single i.v. bolus doses of SJG-136 exhibited a delay in the detection of tumor growth suppression, as described above in the early-stage SF-295 model and in the advanced-stage LOX IMVI models. As shown in Fig. 4, treatment with 178, 267, or 400 \( \mu g/\text{kg/day} \) (total dosages: 0.18 to 0.40 mg/kg = 0.53 to 1.2 mg/m\(^2\)) required ~3 days to confer measurable degrees of tumor growth arrest and 7 days to confer the maximum tumor regression. Although single dose treatments produced prominent effects upon tumor mass, tumor regrowth recurred promptly within the next 4 days, and hence, tumor growth delay indices for the single dose treatments are inferior to those associated with the multiple treatment regimens observed in this tumor xenograft model. Because 400 \( \mu g/\text{kg/day} \) is the maximum-tolerated dose for single bolus administration of this agent (600 \( \mu g/\text{kg/day} = LD_{10} \)), these results demonstrate that a multiple day treatment regimen is more effective in sustaining growth suppression and/or tumor regression than a single bolus regimen. It is noteworthy that 5-day continuous infusions are highly effective in the LOX IMVI tumor model with minimum %C levels of 3 and 0%, tumor growth delays of 142 and 386%, and log cell kills of 0.7 and 2.8 (Table 4). Under conditions of testing, the continuous infusion regimen did not appear to confer any greater efficacy than bolus administrations. Nevertheless, continuous...
infusions were highly active and the lower peak plasma concentrations afforded by them may render infusion a useful mode for SJG-136 delivery in man.

**DISCUSSION**

As noted in the companion article (5), SJG-136 (NSC 694501) is a rationally designed compound, chemically novel and biologically unique in its mechanism of action and *in vitro* pattern of activity. In this article, we present evidence that SJG-136 possesses a number of pharmacologically desirable properties. First, the *in vitro* c × t evaluations demonstrate marked cytostatic and/or cytotoxic activity with brief exposures to nanomolar concentrations or longer exposures to low- to subnanomolar concentrations (Fig. 1). Highly sensitive cell lines exhibit growth inhibition in response to <6 hours × 0.010 to 0.063 nmol/L and lethality with ≤6 hours × 0.66 to 1.9 nmol/L. By contrast, the least sensitive cell lines tested require ≥6 hours × 0.18 to 1.0 nmol/L to exhibit growth inhibition and ≥12 hours × 1.1 to 13 nmol/L to exhibit lethality. Overall, the *in vivo* patterns of efficacy observed for SJG-136 corresponds to the respective *in vitro* c × t profiles measured for these cell lines.

SJG-136 exhibits a broad spectrum of *in vivo* antitumor activity with multiple treatment regimens and a relatively wide dose range. SJG-136 is highly efficacious in each of 10 tumor xenograft models studied: LOX IMVI and UACC-62 (melanoma); OVCAR-3 and OVCAR-5 (ovarian carcinoma); MDA-MB-435 (breast carcinoma or melanoma); C-6 and SF-295 (rat glioma and human glioblastoma, respectively); LS-174T (colon carcinoma); HL-60 TB (promyelocytic leukemia); and NCI-H522 (non-small cell lung carcinoma). After i.v. bolus drug delivery in early-stage xenograft models, SJG-136 produced minimum %C values ranging from 0 to 38% with significant growth delays in seven of eight models, as well as multilog cell kills in four of eight early-stage models (Table 1A). SJG-136 was highly effective in the two advanced-stage xenograft models evaluated, with minimum %C values ranging from 0 to 100% with prominent growth delays, multilog cell kills, and multiple tumor-free mice in each model (Table 4). Bolus treatments on a qd5 schedule conferred better efficacy than treatments on a q4d3 schedule in each of the four models tested on both schedules (Table 1A). In addition, the qd5 schedule exhibited a greater range of effective doses, as shown in Table 3 (16 to 120 μg/kg/dose). However, single large bolus dose treatments and 5-day continuous infusions were also very effective (Table 4).

In addition to meeting conventional criteria for efficacy in terms of minimum %C, growth delays, and multilog cell kill, it is important to note the onset and duration of the protective drug effects. As shown in Figs. 2 and 3, the growth suppression effect of SJG-136 does not become prominent for at least 5 days after treatment initiation. However, the
tumor growth suppression or regression achieved persisted over the course of 12 to 38 days in the LOX IMVI model and 20 to 35 days in the SF-295 model. It is also clear from Figs. 3 and 4 that single bolus treatment is active in the LOX IMVI model, but better activity is conferred by repeated daily treatments of the same total dosages extended over 5 days.

It is important also to note that several SJG-136 treatment regimens tested to date have been active in athymic mice, as well as in athymic rats, and that SJG-136 is well tolerated under conditions of optimal dosages. Although SJG-136 and bizelesin confer activity by different mechanisms of biochemical interaction within the minor groove of DNA (interstrand cross-linking of exocyclic N2 groups of the guanine – guanine between opposite strands of DNA for SJG-136 versus interstrand cross-linking of N3 groups of adenine – adenine for bizelesin; refs. 2, 18), a comparison of the in vivo antitumor activities of these two agents reveals additional similarities and differences in efficacy and toxicity. As previously reported by Carter et al. (17), bizelesin conferred 1.1 to 6.7 log cell kills in three of six syngeneic murine tumor models and 1.0 to 3.0 log cell kills in six of eight human tumor xenograft models. In addition, tumor growth delays ranging from 49 to 263% were observed in the human tumor xenografts with evidence of tumor-free survivors in three of eight xenograft models tested at optimal doses. In the current investigation, SJG-136 was observed to confer growth delays of 83 to 783% and 1.9 to 7.2 log cell kills in 6 of 10 different xenograft models and tumor-free survivors in 6 of 10 models. Although neither SJG-136 nor bizelesin produced delayed drug-related deaths as reported by McGovern et al. (19) for the parent structure, CC-1065, bizelesin treatments were accompanied generally by delayed recoveries of drug-induced body weight loss (17). Furthermore, dosage regimens of bizelesin required to confer significant growth delays (124 to 219%) and multilog cell kills (2.6 to 4.6) in the UACC-62 model were accompanied by body weight losses of 25 to 34%. By contrast, the highly effective SJG-136 treatments on the q×5 schedule were accompanied by more limited body weight losses. It is important to note that substantial body weight losses often result from daily i.v. vehicle-control treatments and that, in the current investigation, body weight losses as high as 17.8% were observed. However, net body weight losses attributable to optimal SJG-136 treatments were generally <10%. Thus, SJG-136 is equally if not more efficacious and appears less toxic than bizelesin in several human tumor xenograft model systems.

In the current investigation, the pyrrolobenzodiazepine dimer, SJG-136, was observed to be highly active in vivo in a broad spectrum of human cancer models. SJG-136 was active by i.v. bolus injection on q×1, q×5, and q×4×3 schedules and by 5-day continuous infusion in athymic mice. Under conditions of testing, q×5 bolus administrations conferred the best efficacy. Furthermore, SJG-136 conferred an antitumor effect over a wide dose range in athymic mice: on a q×5 schedule, the maximum-tolerated dose was ~120 µg/kg/dose, and the minimum effective dose was ~16 µg/kg/dose in each of two solid tumor models. In addition, SJG-136 was well tolerated and efficacious in athymic rats bearing C-6 (rat glioma) and LS-174 (human colon cancer) tumor grafts, indicating that the impressive antitumor activity of SJG-136 in the mouse is not unique to a single rodent species. Whether the pharmacodynamics and pharmacokinetics of SJG-136 in the mouse and rat are similar to that of other laboratory animals and possibly man awaits additional pharmacological investigations. However, detailed studies of rodent versus human bone marrow sensitivities have revealed that, in contrast to bizelesin, mouse marrow and human marrow have comparable sensitivity to SJG-136 (20). Thus, the evidence collected to date suggests that the impressive antitumor activity of SJG-136 in the mouse does not reflect an overt difference in species drug tolerance.

Previous studies of likely predictors of clinical activity in agents studied at the NCI indicate that in vivo activity in ≥33% of the models tested is associated with activity in more than one phase II trial (21). SJG-136 exceeds that criterion for interest. Furthermore, the broad spectrum and highly effective antitumor activity observed for SJG-136 in the current and previous reports coupled with in vitro evidence for antitumor activity after brief exposures to low nanomolar concentrations demonstrate that this agent possesses desirable pharmacological properties. The in vitro and in vivo activity observed and reported for SJG-136 in the current and previous reports confirm the importance of expediting more detailed preclinical pharmacology and toxicology evaluations in support of initial clinical studies of this novel, DNA minor groove interstrand cross-linking agent.

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REFERENCES


Michael C. Alley, Melinda G. Hollingshead, Christine M. Pacula-Cox, et al.


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