Preclinical Antitumor Activity and Pharmacological Properties of Deoxyspergualin

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Abstract

A new antibiotic, deoxyspergualin (DSG), demonstrated antitumor activity against L1210 leukemia in mice. The life span of mice bearing either i.p. or s.c.-implanted L1210 increased >150% following i.p. administration of 25 mg/kg DSG on days 1–9. Activity obtained with i.p. bolus treatments was schedule dependent. The tumor burden in mice bearing the s.c. implanted L1210 was reduced by 4–6 log _10 _ units at the end of treatment when DSG was administered every 3 h for 8 injections on days 1, 5, and 9. By contrast, single injections of DSG on days 1, 5, and 9 allowed the tumor burden to increase at least 100-fold during treatment and daily single injections for 9 days reduced the tumor burden by 2 log _10 _ units. The therapeutic advantage for i.p.-implanted L1210 of maintaining plasma concentrations of DSG was indicated further by infusion studies using s.c.-implanted Alzet osmotic pumps. Tumor burden was reduced by 3.5 and 6 log _10 _ units following s.c. bolus treatments every 3 h on day 1 and a 24-h-infusion, respectively. The optimal infusion time for an infusion rate in mice of 179 mg/kg/day appeared to be 72 h. Pharmacokinetic studies following bolus i.v. injection revealed a rapid plasma clearance of parent drug (20.8 ml/min/kg) and a β half-life of approximately 12 min. The bolus dose kinetics was used to predict the steady state plasma concentrations resulting from s.c. infusion; good agreement was observed between predicted values and experimental results. Based on these preclinical data, DSG has been developed to clinical trial. Initial Phase I protocols involve a 120-h infusion schedule.

Introduction

Spergualin is a novel antibiotic isolated from culture broths of _Bacillus laterosporus_ based on growth-inhibitory effects against transformed chick embryo fibroblasts (1). The purified spergualin demonstrated good antitumor activity against both an i.p.- and s.c.-implanted mouse L1210 leukemia subline and the i.p.-implanted mouse EL-4 leukemia, Ehrlich ascites, and Sarcoma 180 (1). Following structural elucidation, synthesis of spergualin and its 15-deoxy analogue, DSG (Fig. 1), was accomplished (2–4). A direct comparison of the antitumor activity of spergualin and DSG, conducted by screening laboratories under contract to the NCI, indicated that both derivatives had similar activity against the i.p.- and s.c.-implanted NCI L1210 leukemia subline but DSG was effective at lower dosage levels (4). These data, as well as the easier synthesis of DSG, guided the NCI in the selection of DSG for development to clinical trial. A recent structure-activity study involving many analogues of spergualin and using a subline of 1,1210 leukemia has shown that DSG is still one of the most active analogues of spergualin (5).

It is the purpose of the present communication to report detailed studies on the antitumor activity of DSG and relate the data to pharmacological properties of the compound; preliminary accounts of some of these studies have appeared (6, 7).

Materials and Methods

Supplies of DSG were either kindly donated to the NCI by the Microbial Chemistry Research Foundation, Japan, or purchased from the Takara Shuzo Co., Ltd., Japan. For the initial studies, the trihydrochloride salt of DSG was dissolved in saline for administration to the mice. A 50-ml/ml solution had a pH of about 4.9. Later studies were performed with the experimental formulation for DSG which consisted of a freeze-dried powder, containing 100 mg DSG trihydrochloride and 100 mg mannitol, reconstituted with 4 ml sterile water. Dilutions of this solution were made with saline. Distilled-in-glass high performance liquid chromatography solvents were obtained from Burdick and Jackson (Muskegon, MI) or Caledon (Ontario, Canada). Deionized, glass-distilled water was obtained from a Corning Model MP-3A automatic water purification system. All buffer salts and chemicals were analytical reagent grade or better.

Mice and Tumors. Mice were obtained through the Animal Genetics and Production Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, and were kept in holding rooms until their weights were appropriate (minimum of 17 and 18 g for females and males, respectively) for tumor transplantation and experimentation. Athymic mice were held in barrier facilities and used when their weights were approximately 23 g. Animals were allowed food and water, _ad libitum_. Tumor lines (the murine P388 and L1210 leukemias, B16 melanoma and M5076 sarcoma and the human MX-1 mammary tumor) were obtained from the Developmental Therapeutics Program tumor repository.

Experimental Chemotherapy. Tests were performed according to published NCI protocols unless noted otherwise (8, 9). For evaluation of the sensitivity of transplantable murine tumors to DSG, BALB/c × DBA/2 F _1_ (hereafter called CD2F _1_ ) mice were implanted with 10⁶ P388 leukemia ascites cells i.p. or 10⁷ L1210 leukemia cells i.p. or s.c., and C57BL/6 × C3H/Fe mice were inoculated i.p. with either 0.5 ml of 1:10 B16 melanoma brei or 10⁶ M5076 sarcoma ascites cells. Tumor implantation day was designated day 0. Treatment (i.p.) with DSG dissolved in saline was initiated 24 h later and was continued either daily for 5 (P388) or 9 (L1210, B16) days or every fourth day through day 13 (M5076). In each experiment, DSG was tested at several dosage levels and each dose was administered to 6–10 mice; at least 30 tumor-bearing control mice per experiment received saline injections. Animals were retained for life span determinations. For evaluation of the sensitivity of the human MX-1 mammary tumor to DSG, 1-mm (10 ocular micrometer units) cubed fragments were implanted under the renal capsule of athymic nude mice (NCr-nu) and _in situ_ measurements of the length and width were taken using a stereoscopic microscope equipped with an ocular micrometer. Test groups contained 6 mice whereas the vehicle control groups contained 16 mice. Treatment was administered i.p. on days 1, 5, and 9 and animals were killed on day 11. At sacrifice, kidneys were excised and _in situ_ tumor graft size was determined. Tumor size was converted to weight using the formula

\[
\text{Tumor wt (mg)} = \frac{\text{Length} \times \text{width}^2}{2}
\]

where length and width were expressed in mm.

The effects of schedule and route of administration on the antitumor activity of DSG using bolus injections (approximately 15 s) were studied...
in both normal mice (8/test group) and mice bearing Li210 leukemia implanted s.c. (10/test group). The experimental formulation of DSG, prepared fresh each day, was administered according to the schedules and routes outlined in Table 2. On each schedule, DSG was administered at the highest and lowest doses indicated in the table as well as several intermediate doses (ratio of consecutive doses, 0.5). Injection volume per mouse was 0.5 ml for i.p. and p.o. administration and 0.2 ml for i.v. administration. Schedule dependency studies were extended to include administration of DSG by continuous infusion using Alzet osmotic pumps (Model 2001; Alza Corporation, Palo Alto, CA). The latter were loaded with a solution of DSG, equilibrated in saline for 18 h at room temperature, and incubated 2 h at 37°C prior to s.c. implantation. Following preliminary dose (and time)-finding studies, female CD2F1 mice (22 g) in experiment 1 (Table 3) were inoculated i.p. with 10⁶ Li210 leukemia cells on day 0. On day 1, mice either were given bolus injections of DSG s.c. every 3 h for a total of 8 injections (10 mice/group) or were implanted s.c. with the Alzet pumps (6 mice/group) containing DSG (164 mg/ml in sterile water). For implantation and removal of the pumps, mice were lightly sedated with chloral hydrate and subjected to supplemental diethyl ether anesthesia as needed. Pumps were removed after 24, 48, 72, or 96 h. In a second experiment, male CD2F1 mice (22-24 g) were inoculated with 10⁵ Li210 leukemia cells on day 0 and treatment was initiated on day 3. The mice received either daily s.c. bolus injections for 5 days or s.c. infusions for 48, 72, or 96 h. The presence or absence of leukemia in dying mice was assessed on the basis of the presence or absence of ascites and/or splenomegaly at necropsy. DSG toxicity also was assessed by changes in mean animal weight without pumps between days 0 and 6. Each experiment was internally controlled with untreated mice bearing tumor burdens ranging from about 10⁶ to 10⁸ cells to permit comparisons on the basis of estimated log₁₀ change in tumor burden at the end of treatment (10).

Evaluation of Anticancer Activity. For life span assays with the murine tumors, antitumor activity was assessed on the basis of percentage of ILS and net logs of tumor cell kill. Median life spans were calculated from grouped median survival times and percentage of ILS was calculated as

\[
\frac{100 \times \text{Median survival time of treated mice}}{\text{Median survival time of control mice}} - 100
\]

DSG was considered effective in a tumor model if it reproducibly gave ILS > 25%. Calculations of net logs of tumor cell kill were made from the tumor-doubling time which was determined from an internal tumor titration (10). Any animal without gross signs of tumor burden surviving more than 60 days post-tumor implantation was considered cured and was excluded from calculations of percentage of ILS and tumor cell kill.

For the xenograft model, antitumor activity was assessed on the basis of percentage of treated versus control (% T/C) calculated from changes between the initial (day 0) and final (day 11) mean tumor weights according to the formula

\[
\% \text{T/C} = \frac{100 \times \text{Change in mean treated tumor wt (day 11 – day 0)}}{\text{Change in mean control tumor wt (day 11 – day 0)}}
\]

A T/C value indicative of activity was < 20%.

Pharmacokinetics. The pharmacokinetics of DSG in plasma of mice was determined after bolus administration of the compound at 6 mg/kg i.v. and after infusion at approximately 100 mg/kg/day for 3 days via s.c. implanted Alzet osmotic pumps. For the bolus pharmacokinetics, male CD2F1 mice (24-28 g) were housed in individual suspended stainless steel cages. Food and water were allowed ad libitum. Three animals were sacrificed at each of 13 time intervals ranging from 2 to 120 min. The mice for the 2-min interval were sacrificed by decapitation and the blood was collected through a funnel rinsed with 10% EDTA into a 3-ml EDTA Vacutainer blood collection tube (Becton-Dickinson, Rutherford, NJ). The remaining mice were anesthetized with diethyl ether and blood was collected by heart puncture. For infusion pharmacokinetics, male CD2F1 mice (25.6-28.9 g) were anesthetized with diethyl ether. The nape of the neck was swabbed with 70% ethanol and the filled pumps inserted through a 1-cm incision into the lumbar area. The incision was closed with Michelle wound clips. The concentration of DSG in the dosing formulation was confirmed on the day of administration. After surgery, the mice were housed in conventional plastic cages and allowed food and water ad libitum.

Three animals were sacrificed at each of 9 time intervals ranging from 1 to 54 h and 5 mice were sacrificed at 72 h after pump implantation. Blood was collected from anesthetized mice by heart puncture and transferred to EDTA Vacutainer blood collection tubes. Pumps were removed from the animals sacrificed at 72 h and the remaining dose was quantitated to confirm delivery of the desired dose. For both studies, plasma was obtained by centrifugation and kept on ice. Plasma samples were analyzed on the day of collection or were stored at 4°C if analyzed within 24 h or at ~20°C if analyzed beyond 24 h.

Analysis of DSG in the plasma was conducted as reported previously (7). Aliquots of plasma (200 μl) were treated with 70% perchloric acid (8 μl), placed on ice for 30 min, and centrifuged at 15,000 rpm for 5 min. DSG in a portion of the supernatant (44 μl) was derivatized with benzoin by addition of 12 μl 0.2 M sodium sulfite/0.1 M β-mercaptoethanol, 13 μl 4 M benzoin in methyl Cellusolve, and 7 μl 10 M potassium hydroxide. Following heating in boiling water for 4 min and cooling in ice water for 1 min, 13 μl 4 M hydrochloric acid/0.6 M Tris-HCl buffer (pH 9.2) were added. The resulting solution was analyzed for derivatized DSG by a reversed phase high performance liquid chromatographic assay using a Hamilton Prp-1 10-μm poly(styrene divinylbenzene) column and a 0.025 M disodium hydrogen phosphate (pH 8.5)/acetonitrile solvent system (53:47) at a flow rate of 1.5 ml/min. The column eluent was monitored at 418 nm with excitation at 325 nm, a time constant of 6 s, and a range setting of 0.1 μA on a Schoeffel FS 970 spectrophotometer. The plasma data fitted with a least squares MLAB program (11). Plasma clearance (CL) following bolus administration was calculated as

\[
\frac{\text{Dose (μg/kg)}}{\text{AUC (μg/ml × min)}} - \frac{\text{AUC (μg/ml × min)}}{\text{AUC (μg/ml × min)}} - \frac{\text{AUC (μg/ml × min)}}{\text{AUC (μg/ml × min)}} - \frac{\text{AUC (μg/ml × min)}}{\text{AUC (μg/ml × min)}}
\]

in which AUC is the area under the curve. Using the parameters calculated from the single dose biexponential fit, the infusion kinetics was predicted using the equation (12)

\[
\text{Kp} = \frac{\text{Ko}}{\text{CL} \left[ 1 + \left( \frac{\beta - K}{\alpha - \beta} \right) e^{-\alpha t} + \left( \frac{K - \alpha}{\alpha - \beta} \right) e^{-\beta t} \right]}
\]

where \(Kp\) is plasma concentration, \(Ko\) is infusion rate, CL is plasma clearance, \(\alpha\) is phase rate constant, \(\beta\) is phase rate constant, and \(K\) is plasma clearance/volume of the central compartment.

RESULTS

Spectrum of Antitumor Activity. The therapeutic effectiveness of DSG against both i.p.- and s.c.-implanted Li210 leukemia is documented in Table 1. Good efficacy was observed over a wide dosage range and was independent of the tumor implant site. A 25-mg/kg dose administered i.p. on days 1–9 produced ILS values greater than 150% in both models, and good antitumor activity (ILS > 50%) was observed over a 32-fold dosage range. Activity was associated with little or no body weight loss. Similar results were observed in at least three additional studies (data not included). DSG also demonstrated activity against a...
second leukemia, the P388 leukemia. In two experiments, optimal ILS values of 72 and 65% were obtained following i.p. administration of 12.5 and 25 mg/kg DSG, respectively, on days 1-5 (data not shown). No reproducible antitumor activity was observed against either the murine B16 melanoma and M5076 sarcoma or the human MX-1 mammary tumor xenograft under the assay conditions described in "Materials and Methods."

Schedule and Route Dependency. Additional studies using the s.c. implanted LI210 leukemia model were conducted to evaluate the effects of schedule and route of administration on the antitumor activity of DSG (Table 2). In the initial experiment, the two q3h × 8 schedules did not reach a toxic dose level and testing on those schedules was repeated, along with the matching single injection per day regimens (day 1 only and days 1, 5, and 9). With i.p. treatment, a distinct therapeutic advantage was noted with the q3h × 8, days 1, 5, and 9 schedule. The optimal dose of 10.5 mg/kg (total drug dose, 252 mg/kg) reduced the tumor burden at the end of treatment by 4-6 log10 units and increased median survival times by more than 150%. In contrast, a single injection on each of the same treatment days (days 1, 5, and 9) produced a maximum ILS of about 40% and allowed the tumor burden to increase at least 100-fold during treatment. The total dose that could be administered without acute lethality was reduced by 75%. Single daily injections for the same treatment period (days 1-9 regimen) produced an effect between those obtained with the two intermittent schedules: a 2 log10 reduction in the viable tumor cell population was achieved during treatment with a total dose of 63 mg/kg; and life span was increased by about 110%. The therapeutic benefit from multiple injections per day compared to a single injection also was apparent, but less marked, when treatment was restricted to day 1 only. At toxic dose levels on all i.p. schedules, lethality was acute with mice usually dying within 30 min of treatment. With i.v. and p.o. treatments, acute lethality was noted at dose levels lower and higher, respectively, than those for i.p. treatment. The optimal i.v. dose administered for 5 consecutive days reduced tumor burden by about 1 log10 unit while the optimal p.o. dose given for 9 days held the tumor cell population static only during treatment.

The demonstration that the q3h × 8 schedules were more efficacious than single bolus treatments per day suggested that sustaining the plasma concentration of DSG improved the therapeutic response. This possibility was explored further by treating mice bearing i.p.-implanted LI210 with a continuous infusion of DSG (Table 3). In the first trial, the optimal treatment using the q3h × 8 schedule delivered a total dose of 252 mg/kg in 24 h and reduced the tumor burden at the end of treatment by 3.5 log10 units. A total dose of 179 mg/kg (higher dose not possible due to limitations of drug solubility and infusion rate) administered by infusion in the same time period provided an additional 2.5 log10 units of cell kill, bringing the total reduction in tumor burden to 6 log10 units. The best therapeutic response was achieved by infusing 179 mg/kg/day for 3 days; this 72-h infusion produced an approximate 7 log10 reduction in the viable tumor cell population. No additional therapeutic effect was obtained by extending the infusion time to 96 h probably due to increased toxicity to the mice. Two none leukemias (24 and 48 h after the 96-h infusion was terminated) were noted, and animals lost weight (approximately 20% between day 0 and day 6). In the second trial, the activity of DSG was evaluated against a more advanced tumor, and a

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Route</th>
<th>Dose Range (mg/kg/4 h)</th>
<th>Optimal dose (mg/kg/4 h)</th>
<th>Total dose (mg/kg)</th>
<th>ILS* (%)</th>
<th>Approximately Log10 change in tumor burden at end of treatment</th>
<th>Tumorous survivors/total</th>
<th>Normal survivors/total</th>
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</thead>
<tbody>
<tr>
<td>Experiment 1 Day 1 only</td>
<td>i.p.</td>
<td>126-1.9</td>
<td>31.5</td>
<td>31.5</td>
<td>11</td>
<td>-1</td>
<td>0/10</td>
<td>8/8</td>
</tr>
<tr>
<td>q3h, Days 1-5</td>
<td>i.p.</td>
<td>50-4.0</td>
<td>25.2</td>
<td>126</td>
<td>69</td>
<td>-2</td>
<td>0/10</td>
<td>8/8</td>
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<tr>
<td>q3h, Days 1-9</td>
<td>i.p.</td>
<td>56-0.2</td>
<td>14</td>
<td>126</td>
<td>111</td>
<td>-2</td>
<td>0/10</td>
<td>4/8</td>
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<td>HNTD:</td>
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<td></td>
<td></td>
<td>7</td>
<td>63</td>
<td>107</td>
<td>-2</td>
<td>0/10</td>
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<tr>
<td>q4d, Days 1, 5, 9</td>
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<td>21</td>
<td>63</td>
<td>41</td>
<td>+2</td>
<td>0/10</td>
<td>7/8</td>
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<tr>
<td>q3h, Days 1, 5, 9</td>
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<td>10-0.1</td>
<td>10.5</td>
<td>252</td>
<td>153</td>
<td>-4</td>
<td>0/10</td>
<td>8/8</td>
</tr>
<tr>
<td>Day 1 only</td>
<td>i.v.</td>
<td>31.5-1.0</td>
<td>7.9</td>
<td>7.9</td>
<td>6</td>
<td>0</td>
<td>0/10</td>
<td>8/8</td>
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<td>q4d, Days 1-5</td>
<td>i.p.</td>
<td>12-0.2</td>
<td>6.3</td>
<td>31.5</td>
<td>57</td>
<td>-1</td>
<td>0/10</td>
<td>8/8</td>
</tr>
<tr>
<td>q4d, Days 1-9</td>
<td>p.o.</td>
<td>224-1.8</td>
<td>56</td>
<td>504</td>
<td>87</td>
<td>0</td>
<td>0/10</td>
<td>7/8</td>
</tr>
</tbody>
</table>

* Median survival times of tumorous control mice in experiments 1 and 2 were 9.8 and 9.2 days, respectively.

** Log cell kill calculations based on dying mice only. Values rounded to the nearest whole number except for 0.5 values.

*** q4d, daily; q4d, every 4 days; q3h, every 3 h for a total of 8 injections/day; HNTD, highest nontoxic dose (highest dose which resulted in at least seven of eight 60-day survivors among the normal mice).
ANTICANCER ACTIVITY OF DEOXYSPERGUALIN

Table 3 Therapeutic efficacy of DSG administered by s.c. infusion

CD2F1 mice were inoculated i.p. with 10^6 (experiment 1) or 10^5 (experiment 2) L1210 leukemia cells on day 0. The experimental formulation of DSG was administered to the mice either as s.c. bolus injections or s.c. infusions using Alzet pumps starting on day 1 (experiment 1) or day 3 (experiment 2).

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>Dose (mg/kg/day)</th>
<th>Total dose (mg/kg)</th>
<th>Survival time (days)</th>
<th>ILS (%)</th>
<th>Approximate log10 change in tumor burden at end of treatment</th>
<th>Nonleukemic deaths/total</th>
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<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Controls (untreated)</td>
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<td></td>
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<td>q3hx8 on day 1</td>
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<tr>
<td></td>
<td>31.5</td>
<td>252</td>
<td>13.0</td>
<td>64</td>
<td>−3.5</td>
<td>1/10</td>
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<tr>
<td></td>
<td>63</td>
<td>504</td>
<td>2.1</td>
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<td>Toxic</td>
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<tr>
<td>24 h</td>
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<td>15.7</td>
<td>98</td>
<td>−6</td>
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<tr>
<td>48 h</td>
<td>179</td>
<td>358</td>
<td>16.0</td>
<td>102</td>
<td>−5</td>
<td>0/6</td>
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<tr>
<td>72 h</td>
<td>179</td>
<td>537</td>
<td>19.0</td>
<td>140</td>
<td>−7</td>
<td>0/6</td>
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<tr>
<td>96 h</td>
<td>179</td>
<td>716</td>
<td>19.0</td>
<td>140</td>
<td>−6</td>
<td>2/6</td>
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<td>Controls (untreated)</td>
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<td>qd, days 3–7</td>
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<td>15.3</td>
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<td></td>
<td>45</td>
<td>225</td>
<td>14.8</td>
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<td><strong>Infusion</strong></td>
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<tr>
<td>96 h</td>
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<td>680</td>
<td>11.3</td>
<td>34</td>
<td>Toxic</td>
<td>4/6</td>
</tr>
</tbody>
</table>

* Absence of ascites and/or splenomegaly in dying mice at necropsy.

Pharmacokinetic Findings. The plasma disappearance of DSG in mice following a 6-mg/kg i.v. bolus injection is illustrated in Fig. 2. The data from individual mice were fitted with a least squares MLAB computer program (11). A monoexponential fit (broken line) gave the equation

\[ C_p = 16.0e^{-0.061t} \]

with a correlation coefficient \( r \) of 0.86, while a biexponential fit (solid line) gave the equation

\[ C_p = 15.1e^{-0.37t} + 14.4e^{-0.06t} \]

with \( r = 0.96 \). Thus, the plasma disappearance of DSG more closely fits a two-compartment model. The area under the curve for the biexponential fit was 288.8 µg/ml x min with 0.82% beyond 80 min and the plasma clearance was 20.8 ml/min/kg. The α and β half-lives were 1.9 and 11.6 min, respectively. The steady state concentration of DSG achieved during a s.c. infusion of 100 mg/kg/day for 3 days was approximately 3 µg/ml (Fig. 3).

DISCUSSION

The antibiotic, DSG, has demonstrated good reproducible antitumor activity against the murine L1210 leukemia in mice. With daily i.p. administration, activity was observed against both the i.p.- and s.c.-implanted tumor over a wide dosage range. Activity was dependent on schedule, with both the total dose administered per day and the length of treatment being contributing factors. With bolus i.p. treatment on day 1 or days 1, 5, and 9 (Table 2), greater therapeutic efficacy was observed when mice received multiple injections of DSG per day instead of a single treatment and activity was related directly to the
total dose the mice tolerated in 1 day. For example, with a single treatment on day 1, the optimal therapeutic dose was 31.5 mg/kg; higher doses caused death within 30 min implicating peak plasma levels as the cause of lethality. The same optimal dose could be administered every 3 h on the same day without increased lethality suggesting rapid plasma clearance of the compound. Similar observations regarding the total dose tolerated were made for single and multiple treatments per day when mice received three courses of treatment 4 days apart. Although the same total dose was tolerated following three courses of multiple injections per day (q3h x 8, days 1, 5, and 9) as after one course of multiple injections (q3h x 8, day 1), efficacy was increased dramatically with the three course treatment. The peak plasma effect on lethality also was noted following i.v. bolus administrations. In both tumorous (experiments summarized in Table 2) and nontumorous mice (13), lethal i.v. doses of DSG caused immediate death. No cumulative toxicity was observed as similar single doses per day were tolerated whether administered on one day or on five consecutive days. The relatively rapid clearance of DSG suggested by the greater antitumor activity observed with the every-3-h schedules was confirmed by the pharmacokinetic studies in mice that demonstrated a terminal half-life of only 12 min after an i.v. bolus injection of DSG.

While multiple doses per day were more efficacious than a single dose, presumably due to increased exposure time of the tumor cells to effective concentrations of DSG, even greater activity was observed when plasma levels of DSG were maintained for 72 h by s.c. constant infusion (Table 3). Predictions were made for the expected steady state plasma concentrations of DSG resulting from a s.c. infusion based on the i.v. bolus dose kinetics. The predicted plasma curve (Fig. 3) corresponded very well with the actual data collected from a s.c. infusion of 100 mg/kg/day to mice, illustrating that the Alzet osmotic pump placed s.c. is a very good substitute for an i.v. infusion with this particular compound. Such data suggest that bolus kinetic data in humans could be used to predict the steady state levels expected from a constant infusion in humans.

The mechanism of action of DSG has not been defined. However, Kunimoto et al. (14) demonstrated that the antiproliferative effects in vitro of the close analogue, spergualin, were dependent on the amine oxidase concentration in the incubation medium. Studies with DSG also have shown that metabolic activation is required in order for the cytotoxic effects of DSG to be manifested in vitro and that the product of the activation is probably an aldehyde (15). Oxidation to toxic aldehydes in vitro also is characteristic of the natural polyamines, spermine and spermidine (16). Studies are in progress to determine whether the aldehyde is the active antitumor species in vivo. Its formation in vivo is likely because infusions of DSG in dogs caused a toxic cystitis typical of aldehydes (e.g., acrolein formed from cyclophosphamide) (17). This observation suggested that mesna, a thiol compound with the ability to inhibit the urotoxicity of cyclophosphamide by combining with acrolein, may be useful in protecting against the cystitis induced by DSG. Therefore, its effect on the activity of DSG is currently being explored preclinically.

DSG may affect the immune system inasmuch as data have been published to indicate that spergualin can exert both immunostimulating (18) and immunosuppressive effects (19).

DSG has been developed to clinical trial by the NCI based on the efficacy of the compound against L1210 leukemia. A 120-h constant infusion was chosen for the initial Phase I protocols based on the preclinical therapeutic and toxicology studies. In addition to the therapeutic advantage of an infusion schedule demonstrated in the preclinical leukemia model, dose-related neurotoxicity observed after bolus administration in beagles was not observed after continuous infusion (17).

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