Encapsulation of the Topoisomerase I Inhibitor GL147211C in Pegylated (STEALTH) Liposomes: Pharmacokinetics and Antitumor Activity in HT29 Colon Tumor Xenografts

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ABSTRACT

The topoisomerase I inhibitor GL147211C [7-[(4-methylpiperazino)methyl]-10,11-(ethylenedioxy)-(20S)-camptothecin trifluoroacetate], a camptothecin analogue, has significant activity in tumor cell cytotoxicity assays in vitro and antitumor activity in both animal tumor models and human patients. Its toxicity is significant, however, effectively limiting the amount of drug that can be administered and its clinical utility. To determine whether the therapeutic index of GL147211C could be improved, the drug was encapsulated in long-circulating, pegylated (STEALTH) liposomes (SPI-355). The pharmacokinetics and antitumor activity of SPI-355 were compared to those of nonliposomal GL147211C. The plasma pharmacokinetics of SPI-355 in rats were typical of those of other pegylated liposomal formulations, with significantly increased blood circulation time; the dose-corrected area under the curve and Cmax of SPI-355 (10 mg/kg) were 1250- and 35-fold higher, respectively, than those of nonliposomal GL147211C (8.72 mg/kg). The comparative antitumor activity of SPI-355 and nonliposomal GL1472211C was evaluated in nude mice implanted with HT29 colon carcinoma xenografts. SPI-355 was 20-fold more effective than GL147211C in inhibiting tumor growth (1 mg/kg SPI-355 and 20 mg/kg GL147211C) and produced durable complete remissions of tumors at well-tolerated dose levels that were >5-fold lower than the maximally tolerated dose of GL147211C, which induced no durable complete responses. Signs of toxicity were similar between the two drugs, but liposome encapsulation increased the toxicity of drug ~4-fold, with increased weight loss and several deaths with SPI-355 (5 mg/kg SPI-355 versus 20 mg/kg GL147211C). Despite the increased toxicity seen with SPI-355, the therapeutic index of the liposomal formulation was increased ~5-fold over that of nonliposomal GL147211C, suggesting that such a pegylated liposomal formulation could demonstrate increased therapeutic index in human patients.

INTRODUCTION

The camptothecin analogue GL147211C [7-[(4-methylpiperazino)methyl]-10,11-(ethylenedioxy)-(20S)-camptothecin trifluoroacetate], provided by Glaxo Research Institute (Research Triangle Park, NC), is a potent and specific inhibitor of DNA topoisomerase I (1, 2). Topoisomerase I inhibitors stabilize the cleavable complex formed by topoisomerase I and DNA and cause single-stranded DNA breaks (1, 3). This DNA damage, however, is not toxic to the cell until DNA synthesis, when DNA replication forks encounter stabilized cleavable complexes and result in double-stranded DNA breaks (3, 4). Because of this significant cell cycle-dependent antitumor activity (1, 3), prolonged exposure to effective drug concentrations is required to maximize the fractional tumor cell kill (3, 5).

The topoisomerase I inhibitor GL147211C has significant activity in both in vitro cytotoxicity tests and in vivo animal tumor models (2, 6). Phase I clinical trials have been published (7–9) using a 5-day infusion per cycle, with a recommended dose of 1.0–1.5 mg/m2/day. Toxicity of the nonliposomal drug (marked weight loss in mice at clinically effective doses and myelosuppression and moderate gastrointestinal toxicity in Phase I clinical trials in humans) is significant, limiting the amount of drug that can be administered and restricting exposure of tumor tissue to effective drug concentrations (1, 7–10).

STEALTH liposomes are liposomes that contain surface-bound polyethylene glycol chains (11–13). Pegylated liposomes exhibit prolonged circulation times by avoiding uptake by the organs of the mononuclear phagocyte system (13–15). Molecules that have been successfully incorporated into pegylated liposomes, such as doxorubicin, have shown marked increase in circulation times and improvement in antitumor activity (16, 17).

This report summarizes the results of a pharmacokinetic study in rats and efficacy studies initiated with xenograft tumors in mice to determine the antitumor activity of GL147211C encapsulated in long-circulating, pegylated liposomes (SPI-355).

MATERIALS AND METHODS

Test Material

GL147211C (GG211 or Lurtotecan; supplied by Dr. Michael Luzzio at Glaxo Research Institute, Research Triangle Park, North Carolina 27709 [M. L.])

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PK and Antitumor Activity

diameter liposomes. The theoretical concentrations in the final formulation.
PEGylated Liposomal glycero-3-phosphoethanolamine glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt.

Table 1 Lipid components of the pegylated liposomes of SPI-355

<table>
<thead>
<tr>
<th>Lipid component</th>
<th>Lipid content (mg/ml)</th>
<th>Molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSPEC</td>
<td>9 (60)</td>
<td>55.4</td>
</tr>
<tr>
<td>MPEG-DSP</td>
<td>3 (20)</td>
<td>5.6</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3 (20)</td>
<td>39</td>
</tr>
</tbody>
</table>

a Values shown are the theoretical concentrations in the final formulation.

SPI-355, hydrogenated soy phosphatidylcholine; MPEG-DSP, N-carbamoyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt.

Pharmacokinetic Study

Animals. Eight male Sprague Dawley rats were obtained from Simonsen Labs (Gilroy, CA) and allowed to acclimate for 5 days prior to study initiation. Rats were housed in conventional hard-bottomed cages with food and acidified water ad libitum under a 12-h light/dark cycle. Experiments were conducted under the auspices of the Institutional Animal Care and Use Committee of SEQUUS Pharmaceuticals, following the 1996 Guide for the Care and Use of Animals in Research.

Study Design. SPI-355 and nonliposomal GL147211C were administered as a single bolus via lateral tail vein at 10 and 8.72 mg/kg, respectively (n = 4 rats per formulation). Blood samples were collected at 3–5, 15, and 30 min and 1, 2, 4, 6, and 24 h postdose. Two 0.20-ml samples of whole blood were extracted in 0.40 ml of either 4°C AcN2 or 25°C acidified AcN (H3PO4), immediately vortexed vigorously for 10 s, and then centrifuged at 13,000 rpm for 5 min at room temperature. Supernatant was removed and immediately frozen at −70°C until assay by HPLC (18). Assay results for the acidified AcN extract (lactone form) and the nonacidified AcN extract were essentially equal. Here, we report values obtained from acidified AcN extract (lactone form).

Data Analysis. Mean drug concentrations in whole blood were plotted against time. The apparent half-lives were calculated as ln(2)/k1, where k1 is the elimination constant of drug from blood. The AUC∞ measurement was determined by the linear trapezoidal rule and extrapolated to infinity by dividing the last measured concentration into the slope of the terminal phase. The maximum concentration achieved (C max) was measured directly.

Efficacy Studies

Animals. Two studies were conducted at Southern Research Institute (Birmingham, AL) on a contract basis. For each study, 80 homozygous nude mice were obtained from Taconic Farms (Germantown, NY) and allowed to acclimate for 7 days prior to initiation of the experiment. Animals were housed in appropriate isolated caging with sterile rodent food and acidified water ad libitum and a 12-h light/dark cycle. Animals were randomized into treatment groups prior to inoculation of tumors based on body weight. Randomization was confirmed based on tumor size immediately prior to initiation of treatment.

Tumors. Tumors were inoculated s.c. by trochar placement of fragments from rapidly growing tumors on donor animals. The human colon cancer cell line HT-29 was used to initiate xenograft tumors.

Monitoring. All animals were observed daily for general well-being throughout the experiments. Animals were weighed prior to inoculation of tumors and at least weekly thereafter. Tumors were measured twice weekly throughout the experiment, beginning 10 days after tumor inoculation. Any animal with a weight loss of ≥15% of the initial starting weight was immediately euthanized, as was any animal with a tumor volume of >4000 mm³. Experiments were conducted under the auspices of the Institutional Animal Care and Use Committees of SEQUUS and Southern Research Institute following the 1996 Guide for the Care and Use of Animals in Research.

Treatment. Animals were randomized to one of seven treatment groups (n = 10 animals each) in each experiment (20 animals in negative control group). Treatment was initiated on day 9 or 10 postinoculation, when the average tumor volume was ~75 mm³ and the tumors were progressively growing. All treatments were administered as i.v. bolus injections, given weekly for 3 consecutive weeks. In study 1, treatment groups were nonliposomal GL1472111C at 6, 15, or 24 mg/kg and SPI-355 at the same dose levels. In study 2, treatment groups were nonliposomal GL1472111C at 20 mg/kg and SPI-355 at 5, 3, 1, 0.5, and 0.1 mg/kg.

Evaluation. Tumor size during and following each experiment was used as the primary evaluation of therapeutic efficacy. Body weight and survival were evaluated to assess toxicity. All tumor-bearing animals were observed following cessation of treatment until they were euthanized, based on criteria above. Experiments were concluded when a majority of control tumors achieved the maximal allowed volume (4000 mm³).

Data Analysis. For each individual, tumor size was measured repeatedly at various time points; thus, these measurements were regarded as correlated information. Because the tumor sizes over time after treatment were of interest, repeated measurement analyses were performed for each data set. From examination of the data, a log transformation seemed reasonable. In this transformation, Y denotes the original tumor measurement, and Z = log(Y + 1). After the data were transformed, repeated measurement analyses were performed for the trans-
Table 3 Pharmacokinetic parameters after a single i.v. bolus of SPI-355 (10 mg/kg) or nonliposomal GL147211C (8.72 mg/kg) in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SPI-355</th>
<th>Nonliposomal GL147211C</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (µg/ml • h)</td>
<td>1853 ± 268</td>
<td>1.49 ± 0.18</td>
</tr>
<tr>
<td>Apparent T1/2 (h)</td>
<td>21.1 ± 4.33</td>
<td>1.58 ± 0.41</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>84.2 ± 11.6</td>
<td>2.44 ± 0.23</td>
</tr>
</tbody>
</table>

diagram

Formulations

**RESULTS**

Pharmacokinetic Study

In rats administered nonliposomal GL147211C, blood concentrations declined in a biexponential manner. The concentration (mean ± SD) at 3–5 min postdose was 2.44 ± 0.23 µg/ml and declined to 0.036 ± 0.016 µg/ml by 6 h postdose (Fig. 1 and Table 2). No GL147211C was detected in blood at 24 h postdose. The AUCmax was 1.49 ± 0.175 µg/ml•h, and the apparent half-life was 1.58 ± 0.41 h (Table 3).

In rats administered SPI-355, blood concentrations of GL147211C also declined in a biexponential manner; at 3–5 min postdose, the concentration was 84.2 ± 11.6 µg/ml, and it declined to 28.1 ± 3.17 µg/ml by 24 h postdose (Fig. 1 and Table 2). The AUCmax of SPI-355 was 1853 ± 268 µg/ml•h, and the apparent half-life was 21.1 ± 4.33 h (Table 3).

Efficacy Studies

**Study 1: HT29 Colon Xenograft.** There were significantly more deaths in the SPI-355 treatment groups than in animals that received the same doses of nonliposomal GL147211C. All animals in the 15 and 24 mg/kg SPI-355 dose groups died after two doses, with most deaths occurring on day 5 after the first dose. Animals in the 6 mg/kg dose group survived all three doses, but 6 of 10 died within 8 days after the third dose. With the exception of one animal in the 24 mg/kg nonliposomal GL147211C group that died after the third dose, all animals survived in the nonliposomal GL147211C treatment groups. Body weight losses reflected the greater toxicity of liposomal SPI-355 (Fig. 2).

Despite its greater toxicity, the 6 mg/kg dose level of SPI-355 showed substantial antitumor activity (log growth rate of −0.026), significantly greater than that displayed by even the highest dose level of nonliposomal GL147211C (log growth rate of 0.0048; P = 0.0022). Tumor growth was significantly inhibited by treatment with 6 mg/kg SPI-355 (Fig. 3), with CR of tumors in all 10 animals by 5 days after the second treatment (Table 4). Tumors did not recur in surviving animals (4 of 10) in this treatment group by the end of study, ~30 days after final treatment.

Nonliposomal GL147211C also caused a significant inhibition of tumor growth (Fig. 3), particularly at a dose of 24 mg/kg, but did not result in as many tumor remissions (three CRs and one PR) as a 4-fold lower dose of SPI-355 (Table 4), and these remissions were not sustained by day 69. At a dose level of 6 mg/kg, free GL147211C caused no tumor remissions and was only minimally effective at inhibiting growth of HT29 tumors (Fig. 3).

**Study 2: HT29 Colon Xenograft.** Because of drug-related toxicity seen in the first study, a repeat study was conducted in this tumor model using lower dose levels in an effort to define the MTD and antitumor activity of SPI-355. Because the activity profile of nonliposomal GL147211C was well defined in first study, only one dose level was used in this study. Additionally, the dose level was decreased from 24 to 20 mg/kg, because one animal died at the higher dose in the first study.

At lower doses in this study, 4 of 10 animals in the 5 mg/kg SPI-355 dose group died of drug-related toxicity after dose 3; one additional animal died of apparently nonspecific causes after the third dose. One of 10 animals in the 3 mg/kg SPI-355 dose group died after dose 2, but death was not considered due
to drug treatment because of absence of any correlating signs of toxicity. All other animals survived the entire study duration, and there were no deaths in the 20 mg/kg nonliposomal GL147211C dose group. Body weight changes were dose-related and correlated with other observations of toxicity (Fig. 4).

Tumor growth was significantly inhibited by all treatment regimens. Treatment with 20 mg/kg nonliposomal GL147211C significantly inhibited tumor growth (log growth rate of 0.011) and was approximately equivalent in its antitumor activity to 1 mg/kg SPI-355 (log growth rate of 0.017; P = 0.091; Fig. 5). At 3 mg/kg, SPI-355 (log growth rate of −0.029) exhibited significantly greater antitumor activity than 20 mg/kg of nonliposomal GL147211C. Treatment with 20 mg/kg nonliposomal GL147211C significantly inhibited tumor growth (log growth rate of 0.011) and was approximately equivalent in its antitumor activity to 1 mg/kg SPI-355 (log growth rate of 0.017; P = 0.091; Fig. 5). At 3 mg/kg, SPI-355 (log growth rate of −0.029) exhibited significantly greater antitumor activity than 20 mg/kg of nonliposomal GL147211C.

**Table 4** Response of HT29 colon cancer xenografts: study 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CR</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0/20</td>
<td>0/20</td>
</tr>
<tr>
<td>GL147211C, 24 mg/kg</td>
<td>3/10</td>
<td>1/10</td>
</tr>
<tr>
<td>GL147211C, 15 mg/kg</td>
<td>2/10</td>
<td>0/10</td>
</tr>
<tr>
<td>GL147211C, 6 mg/kg</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>SPI-355, 24 mg/kg</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SPI-355, 15 mg/kg</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SPI-355, 6 mg/kg</td>
<td>10/10</td>
<td>NA</td>
</tr>
</tbody>
</table>

*CR, elimination of tumor mass until experiment termination; PR, tumor volume of <50% of peak tumor volume for an individual animal; NA, not applicable.*
Body weights of HT29 colon tumor-bearing nude mice from study 2. Data points, mean body weights; bars, SE. Animals were treated with SPI-355 or nonliposomal GL147211C on days 9, 16, and 23 after tumor inoculation. All treatments were associated with transient weight loss, recovered prior to subsequent treatment, with the most significant weight loss in high-dose SPI-355 treatment group.

Table 5 Response of HT29 colon cancer xenografts: study 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CR</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0/20</td>
<td>0/20</td>
</tr>
<tr>
<td>GL147211C, 20 mg/kg</td>
<td>0/10</td>
<td>1/10</td>
</tr>
<tr>
<td>SPI-355, 5 mg/kg</td>
<td>10/10</td>
<td>NA</td>
</tr>
<tr>
<td>SPI-355, 3 mg/kg</td>
<td>7/10</td>
<td>1/10</td>
</tr>
<tr>
<td>SPI-355, 1 mg/kg</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>SPI-355, 0.5 mg/kg</td>
<td>0/10</td>
<td>1/10</td>
</tr>
<tr>
<td>SPI-355, 0.1 mg/kg</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* CR, elimination of tumor mass until experiment termination; PR, tumor volume of <50% of the peak tumor volume for an individual animal; NA, not applicable.

DISCUSSION
The pharmacokinetics of SPI-355 observed here are typical of other STEALTH liposome-encapsulated molecules. Encapsulation of GL147211C into STEALTH liposomes significantly increases its blood circulation time compared to nonliposomal GL147211C, allowing prolonged exposure of tumor tissue to significant drug concentrations. In rats administered SPI-355, the dose-corrected \( C_{max} \) and \( AUC_{0\infty} \) values were 35- and 1250-fold higher, respectively, compared to nonliposomal GL147211C. The decline in blood concentrations of both SPI-355 and nonliposomal GL147211C was biexponential, and the apparent half-life of SPI-355 was ~13-fold longer than that of nonliposomal GL147211C (21.1 \text{ versus} 1.58 \text{ h}, respectively).
The first antitumor activity study with HT29 colon xenografts was performed with dose levels selected from previously published data on the MTD of nonliposomal GL147211C (6). Toxicity was evident after the first administration of SPI-355, with death or euthanasia of all animals in the 24 and 15 mg/kg dose groups by the second weekly drug administration. Despite significant toxicity at the lowest dose (6 mg/kg), SPI-355 demonstrated remarkable antitumor activity in surviving animals, with all 10 HT29 colon tumor-bearing animals having a CR, which was sustained for the duration of the study in the survivors.

The second antitumor activity study established that the MTD of SPI-355 for repeated administration is between 3 and 5 mg/kg. The antitumor activity of 1 mg/kg SPI-355 was significant and equivalent to a 20-fold higher dose of nonliposomal GL147211C. Both 3 and 5 mg/kg SPI-355 were significantly more effective than 20 mg/kg nonliposomal GL147211C. These findings suggest that the therapeutic index of SPI-355 is ~4–5-fold higher than that of nonliposomal GL147211C, as confirmed by the large number of complete and partial antitumor responses in the 3 mg/kg (7 of 10 CRs and 1 of 10 PRs) and 5 mg/kg (10 of 10 CRs) SPI-355 groups. Nonliposomal GL147211C, although demonstrating significant tumor growth delay, failed to produce any CRs and produced only one PR. A recently described conventional, nonpegylated liposomal formulation of GL147211C, NX-211 (19), also increased drug AUC and enhanced antitumor activity, but only at dose levels similar to nonliposomal GL147211C; no data on tumor remissions were provided. This report suggests that encapsulation of GL147211C in pegylated liposomes confers an additional therapeutic advantage.

The increased circulation time associated with pegylated liposomal encapsulation of SPI-355 likely allows prolonged exposure of tissues to significant drug concentrations, as seen with other cytotoxic drugs encapsulated in pegylated liposomes (16). The prolonged exposure increases the cell cycle-specific cytotoxicity of the drug and may account for the large number of cures among study animals (3, 5). Although increased toxicity (4-fold) of SPI-355 was observed, the improvement in antitumor activity (20-fold) and therapeutic index (5-fold) of this formulation over the nonliposomal GL147211C warrants further study.

REFERENCES

Encapsulation of the topoisomerase I inhibitor GL147211C in pegylated (STEALTH) liposomes: pharmacokinetics and antitumor activity in HT29 colon tumor xenografts.

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