A Pilot Clinical/Pharmacological Study of the Protein Kinase C-specific Inhibitor Safingol Alone and in Combination with Doxorubicin

Gary K. Schwartz,1 David Ward, Leonard Saltz, Ephraim S. Casper, Tara Spiess, Eileen Mullen, Jim Woodworth, Robert Venuti, Peter Zervos, Anne-Marie Storniolo, and David P. Kelsen

Department of Medicine, Division of Solid Tumor Oncology, Gastrointestinal Oncology Section, Memorial Sloan-Kettering Cancer Center, New York, New York 10021 [G. K. S., L. S., E. S. C., T. S., E. M., D. P. K.], and Eli Lilly Research Lab, Indianapolis, Indiana 46285 [D. W., J. W., R. V., P. Z., A.-M. S.]

ABSTRACT

We performed a pilot clinical trial with safingol (L-threo-dihydrosphingosine), a protein kinase C-specific inhibitor that potentiates the effect of doxorubicin (DOX) in tumor-bearing animals. Safingol was initially administered as a 1-h infusion at escalating doses. Fourteen days later, patients received the same dose of safingol in combination with a fixed dose of DOX. The combination was repeated at 3-week intervals. Safingol dose levels ranged from 15 to 120 mg/m². The plasma levels achieved at the final dose level were comparable to those associated with potentiation of DOX in animals. The mean Cmax and area under the curve for safingol at the 120 mg/m² dose level were 1040 ± 196 ng/ml and 1251 ± 317 mg × h/ml, respectively. The mean plasma half-life for safingol was 3.97 ± 2.51 h, the mean estimated clearance was 3140 ± 765 ml/min, and the mean volume of distribution was of 995 ± 421 liters. Coadministration of a fixed dose of DOX did not significantly change the pharmacokinetics of safingol, nor did increasing doses of safingol significantly affect the pharmacokinetics of DOX. Minor responses were observed in three patients with pancreatic cancer and one patient with angiosarcoma of the scalp. This pilot Phase I study indicates that the protein kinase C inhibitor safingol can be given safely with 45 mg/m² of DOX at a dose that is potentially pharmacologically active without dose-limiting toxicity.

INTRODUCTION

PKC is a phospholipid-dependent serine/threonine kinase that plays a pivotal role in many of the signal transduction events related to tumorigenesis. Activation of cell surface receptors by extracellular molecules such as growth factors and hormones can stimulate phosphatidylinositol turnover, leading to elevated levels of inositol triphosphate and sn-1,2-diacylglycerol, the endogenous ligand that activates PKC (1, 2). These extracellular signaling molecules, as well as their receptors, are altered in their effect by cellular oncogenes, and diacylglycerol is known to be elevated in cells transformed by ras, sis, and src (3). In addition, activation of PKC leads to the expression of nuclear proto-oncogenes (e.g., myc, fos, and fms) associated with cellular proliferation. PKC is activated by phorbol esters, such as phorbol myristate acetate, which are potent tumor promoters in mouse skin (4). Increased tumorigenicity also correlates with overexpression of certain PKC isoforms in NIH 3T3 cells inoculated into nude mice (5). The involvement of PKC in so many aspects of aberrant growth regulation suggests that the modulation of PKC activity might be an effective target for anticancer therapy.

Safingol (Fig. 1), the L-threo enantiomer of dihydrosphingosine, is a PKC-specific inhibitor that inhibits PKC enzyme activity in micromolar concentrations (6). In vitro studies of safingol have demonstrated reversal of multidrug resistance in DOX-resistant cell lines (7, 8). Safingol has also been shown to increase the activity of DOX and other chemotherapeutic agents, including mitomycin C, by enhancing chemotherapy-induced apoptosis, even in tumor cell lines that were resistant to chemotherapy by virtue of a mutation in p53 (6). In vivo, as a single agent, safingol has shown little direct antitumor activity. However, in a series of murine tumor models and human tumor xenografts, safingol has been shown to significantly modulate the antitumor effect of DOX and cisplatin (9, 10). This was achieved without an increase in chemotherapy-induced bone marrow suppression or major organ toxicity (i.e., ear or kidney). In these studies, nontoxic serum levels of safingol could be attained at concentrations associated with inhibition of PKC enzyme activity (11).

Because the in vivo activity of safingol appeared to be greatest when combined with chemotherapy and because this was associated with established serum safingol levels, we performed a pilot clinical study of safingol and DOX with pharmacological end points designed to determine whether we could

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1 To whom requests for reprints should be addressed, at Department of Medicine, Division of Solid Tumor Oncology, Gastrointestinal Oncology Section, Memorial Sloan-Kettering Cancer Center, New York, NY 10021. Phone: (212) 639-8324; Fax: (212) 717-3320.

2 The abbreviations used are: PKC, protein kinase C; DOX, doxorubicin; PT, prothrombin; PTT, partial thromboplastin; CBC, complete blood count; DLT, dose-limiting toxicity; HPLC, high-performance liquid chromatography; AUC, area under the curve; HNTD, highest nontoxic dose.
achieve nontoxic levels of safingol in serum associated with chemopotentiati
tion of DOX in animals.

PATIENTS AND METHODS

Patients. Patients treated as part of this clinical trial had to meet the following criteria. All patients had to be ≥18 years of age with histologically confirmed carcinoma by the Pathology Department of Memorial Hospital. Patients had to have cancer refractory to standard therapy (or for which there was no standard therapy) and not curable by surgery or radiation therapy. A Karnofsky performance status ≥60% with a life expectancy of at least 12 weeks was required. Previously treated patients were accepted. They may not, however, have received myelosuppressive chemotherapy or radiation therapy to major bone marrow-containing areas within the previous 4 weeks (6 weeks for prior nitrosourea or mitomycin C), and they must have recovered from the marrow toxic effects of prior chemotherapy and radiation.

All patients must have had a WBC count ≥4000/µl, a total neutrophil count ≥1500/µl, a platelet count ≥150,000/µl, and a hemoglobin ≥10 g/dl, and a normal PT/PTT prior to starting therapy. Normal renal function (serum creatinine ≤1.5 mg/dl) and normal hepatic function (serum bilirubin ≤1.5 mg/dl; serum aspartate aminotransferase and alkaline phosphatase levels ≤2.5 times the upper limit of normal) were required. Patients with documented bone metastases and an elevated alkaline phosphatase >2.5 times the upper limit of normal were eligible for the study if all other liver function tests were within the above-specified limits. A normal resting gated pool heart scan with an ejection fraction ≥50% was required. Patients were required to have no prior history of cardiac arrhythmia requiring therapy (other than chronic atrial fibrillation). Patients with a history of a myocardial infarction within 6 months prior to study entry were excluded. Prior DOX treatment was allowed as long as the prior lifetime cumulative dose of DOX did not exceed 280 mg/m². Good peripheral venous access was required; otherwise central access via a Mediport or Broviac catheter was required. Patients with evidence of hemolysis or a history of non-drug-induced hemolysis (e.g., spherocytosis) were excluded. Females of child-bearing potential were required to have a negative pregnancy test for females. Radiological studies with measurement of the tumor indicator lesion(s) were performed as clinically indicated. Pretreatment gated pool heart scan at rest and a 12-Lead EKG were required.

Safingol Administration. Safingol is the non-proprietary name for the L-threo enantiomer of dihydrosphingosine. The chemical name is (2S,3S)-2-amino-1,3-octadecanediol; the Eli Lilly Research Laboratory code number is SPC-100270; and safingol is a white to off-white crystalline solid with a molecular weight of 301.50. The molecular formula is C₁₈H₃₉N₂O₂.

Safingol was supplied by Eli Lilly Research Lab (Indianapolis, Indiana) as a 0.5% (5 mg/ml) emulsion, which was diluted with 5% dextrose in water to achieve a 0.5 mg/ml solution for administration. The lipid emulsion contained soybean oil, Pluronic F-68 (NF), cholesterol (NF), α-tocopherol (USP), glycerin (USP), water, and HCl. The vials were stored under at 2–8°C and were for single-use only. The appropriate amount of safingol was diluted in 5% dextrose in water to achieve the desired concentration. The diluted emulsion was stored at room temperature and was administered within 12 h of dilution when stored diluted in either polyvinyl chloride bags or glass bottles. To ensure adequate mixing, the diluted emulsion was shaken just prior to the start of administration. Adriamycin PFS, the brand of DOX manufactured by Pharmacia Laboratories (Columbus, Ohio), was supplied for use in this study.

As shown in Fig. 2, the first cycle of treatment for each patient began with the administration of safingol alone over 1 h at the first dosing visit (day 1, dose 1). Two weeks later (day 14), if the patient did not have any ongoing toxicity, the patient received the second administration of safingol as a 1-h infusion at the same dose (dose 2) over 1 h. Beginning 1 h after completion of the safingol infusion, DOX was delivered via a 5-min i.v. push.

Because safingol emulsion had not previously been given to humans, the first six patients who received the first dose level (15 mg/m²) were hospitalized for 24 h following their first two
safingol treatments. In addition to pre-dose assessments at the start of each cycle of treatment, vital signs were measured every 15 min during the safingol infusion, 1 h after completion of the safingol infusion (just prior to the administration of DOX), and hourly thereafter for the remaining observation period in the clinic (up to 6 h post-safingol infusion, for the first two safingol visits; up to 3 h post-safingol infusion at other cycle dosing visits). Physical exam, CBC with differential, platelets, PT, PTT, biochemical screening profile, blood urea nitrogen, creatinine, electrolytes, and urinalysis were also obtained 24 h after safingol administration for the first two safingol dosing visits.

Because safingol administration was associated with hemolysis in the animal models, extensive hemolysis assays were required for all patients. These included: serum hemoglobin, hematocrit, red cell morphology, serum haptoglobin, and urine for hemosiderin prior to dosing at each dosing visit and at fixed intervals during and following safingol administration. To prevent mechanical hemolysis during blood drawing for these assessments, a 21-gauge (or larger) needle was used. In view of the lack of an National Cancer Institute common toxicity scale for hemolysis, it was necessary to devise our own grading scale (Table 1). If a patient developed hemolysis after a given cycle of treatment in the study (including the administration of safingol alone at the first dosing visit), a series of modifications to the subsequent administration(s) of safingol in that patient were implemented. Patients who developed grade 1 or greater hemolysis through a central or peripheral vein would receive, on subsequent cycles, the safingol infusion only into a central vein at one-half the concentration and twice the volume of the prior regimen. All other nonhemolytic toxicities were graded according to the National Cancer Institute common toxicity criteria.

Additional visits during the first cycle of treatment were scheduled 1 week after dosing with safingol alone and at weekly intervals for 3 weeks after dosing with combination treatment. CBC and platelet count were obtained weekly during the course of therapy. A chest X-ray was obtained each month. Compliance with ongoing birth control in female patients of child-bearing potential was verified weekly and a serum pregnancy test, if indicated, was obtained. A 12-lead EKG was performed 2–3 h after safingol administration for the first three safingol treatments and before administration of any cycle of treatment that resulted in a cumulative life-time dosage of DOX of >300 mg/m² and at the final study evaluation visit. Gated pool heart scan was required prior to any cycle of treatment that resulted in a cumulative DOX dose of >300 mg/m². Tumor measurements with appropriate imaging studies were performed after each cycle of combination therapy.

Individual patients could receive the same dose of safingol and DOX every 21 days unless there were signs of tumor progression, development of a significant toxicity that, in the investigator’s judgment, precluded further therapy, clinical deterioration, or evidence of a decrease in left ventricular ejection fraction of ≥10% from baseline. If toxicity resulting from the administration of safingol alone at the first dosing visit persisted for 2 weeks, patients were not eligible to receive the first administration of safingol in combination with DOX. Depending on the nature of the toxicity, the investigator could withdraw the patient from the study at that time or wait up to 2 weeks for the toxicity to resolve before administering combination treatment.

The dose of safingol received by a given patient was neither increased nor decreased during subsequent cycles. The dose of DOX remain fixed (either at 45 or 60 mg/m²) for each patient regardless of safingol dosage. If the safingol infusion was administered into a peripheral vein, DOX was injected into a different peripheral vein. Both drugs could be administered into the same central line. The dose of safingol was sequentially increased in each cohort of three patients by 100%. If any one patient developed grade 2 toxicity of any type attributed to safingol alone during the first 2 weeks, or if there was an apparent increase in the expected toxicity of DOX following administration of the combination of safingol and DOX, or if the serum levels of safingol approached those associated with toxicity in preclinical animal studies, subsequent increases in the dose of safingol were made according to a modified Fibonacci scale. Each group of three patients was treated and followed for 21 days after administration of combination treatment before the next group of three patients was enrolled.

DLT was defined as the occurrence of grade 4 hematological toxicity, grade 4 nausea and vomiting, grade 2 neurological toxicity, or grade 3 toxicity of any other kind, including hemolysis. If DLT was demonstrated in one or two of three patients,
an additional three patients were enrolled at the same dosage. If no more than two of all six patients encounter DLT, three patients were to be enrolled at the next higher dosage level. If three or more of all six patients develop DLT, then the maximum tolerated dose was exceeded, and three more patients were treated at the next lower dose. The maximum tolerated dose was defined as the dose level at which 0 of 6, 1 of 6, or 2 of 6 patients experienced DLT, with the next higher dosage having at least 3 of 6 patients encountering DLT.

**Pharmacokinetics.** Pharmacokinetics were performed at each of the first two safingol dosing visits. Plasma samples were obtained for HPLC analysis at the following time points in minutes: 60 (just prior to the start of the safingol infusion), 0 (immediately after completion of the 1 h of safingol infusion), 5, 15, 30, 60, 65, 80, and 95 minutes and then 1, 2, 3, 4, 6, and 24 h after safingol infusion.

The method for safingol measurements requires HPLC with fluorescence detection. Briefly, safingol was extracted with N-butyl chloride from human plasma made basic by the addition of 0.1 N sodium hydroxide. The organic layer was transferred to a clean test tube and evaporated to dryness. The residue was reconstituted in methanol and derivatized with o-phthaldialdehyde in the presence of mercaptoethanol for HPLC analysis (12). This method is sensitive to a safingol concentration of 0.036 μg/ml. The method for DOX measurements was performed as described previously (13). The ensuing plasma concentration-time profiles were generated using a noncompartmental method (14).

**RESULTS**

**Patient Population.** As shown in Table 2, 17 patients entered the study (10 males and 7 females). The median age of this group was 59 (range, 29–77), and the median Karnofsky performance scale was 80 (range, 70–90). There was a wide range of tumor types entered into the study. These include: pancreatic (6), gastric (2), colon (3), unknown primary (2), sarcoma (3), and nasopharyngeal (1). Twelve patients had been treated previously with chemotherapy.

**Hematological Toxicity.** Safingol either alone (data not shown) or in combination with 45 mg/m² DOX (Table 3) did not cause dose-limiting hematological toxicity. The dose escalation of safingol with 45 mg/m² of DOX was carried out to a safingol dose of 120 mg/m² before the trial was suspended for lack of sufficient drug supply. As shown on Table 3, with the combination of 120 mg/m² of safingol and 45 mg/m² of DOX, the mean WBC, absolute neutrophil count, and platelet counts were $5.4 \times 10^3/\mu l$, $5.5 \times 10^3/\mu l$, and $263 \times 10^3/\mu l$, respectively. Patients received a mean of 2.5 cycles (range, 1–11) of the safingol and DOX combination. At the 120 mg/m² level, one patient received 11 cycles of safingol (total safingol dose, 1320 mg/m²) in combination with DOX (total DOX dose, 495 mg/m²) without evidence of cumulative hematological or cardiac toxicity. No patient required a dose reduction of either safingol or DOX because of cumulative hematological toxicity at any dose level.

An additional three patients were treated with 15 mg/m² of safingol but with 60 mg/m² of DOX (Table 3). There was no toxicity with safingol alone. However, all three patients experienced dose-limiting leukopenia (mean WBC nadir, $0.4 \times 10^3/\mu l$; range, 0.4–0.6 $\times 10^3/\mu l$) or neutropenia (mean absolute neutrophil count nadir, $0.4 \times 10^3/\mu l$; range, 0.1–0.9 $\times 10^3/\mu l$) with their first cycle of safingol and DOX together. There was also significant thrombocytopenia (mean platelet nadir, 55 $\times 10^3/\mu l$; range, 30–90 $\times 10^3/\mu l$). Two of these three patients had been extensively pretreated with chemotherapy.

**Nonhematological Toxicity.** Nonhematological toxicity was also mild and not dose-limiting with either safingol alone or in combination with DOX. One patient at a safingol dose of 60 mg/m² experienced (when safingol was administered alone) grade I hemolysis as evidenced by a >50% decrease in serum haptoglobin. This patient’s haptoglobin levels decreased from a baseline of 110 mg/dl to 26 mg/dl 6 h following safingol therapy and was fully recovered 72 h later. There was no associated change in reticulocyte count, no evidence of hemolysis on the peripheral smear, no pink discoloration to the plasma, and no evidence of urine hemosiderin. This degree of hemolysis was believed to be related to this patient’s relatively poor venous access. The patient was retreated on day 14, according to the protocol criteria for grade I hemolysis, which included infusion of safingol through a central vein at one-half the concentration (e.g., twice the total volume but the same total dose). Under these conditions, the patient experienced no further evidence of hemolysis. One patient registered to the study with a diagnosis of adenocarcinoma of unknown primary developed intense facial flushing, without other associated toxicity, after receiving only 2–3 mg of safingol. She was taken off study and rendered invaluable for response. Initially, this was believed to be an allergic reaction to safingol that was unrelated to the total safingol dose. Later, the patient had an [111In-pentetreotide scan](https://clincancerres.aacrjournals.org) that was highly positive for somatostatin receptor-positive tumor at the sites of her multiple liver metastases. She, therefore, was subsequently adjudicated as having a neuroendocrine tumor, and the facial flushing was related to her underlying disease rather than directly related to the drug.

**Safingol Pharmacokinetics.** Fig. 3 shows a plot of $C_{\text{max}}$ and AUC for each patient versus safingol dose in mg/m². As shown, up to a safingol dose of 120 mg/m², the increase in $C_{\text{max}}$ and AUC was generally linear with increasing safingol dose. At 120 mg/m², the mean $C_{\text{max}}$ was 1040 ± 196 ng/ml, and the mean AUC was 1251 ± 317 ng × h/ml. There were excellent correlations of 0.82 and 0.87 between the safingol dose in mg/m² and the respective $C_{\text{max}}$ and AUC of the drug.

Analysis of the AUCs for DOX indicate that increasing doses of safingol did not affect the AUC of a 45 mg/m² i.v. bolus of DOX (Table 4). For example, the mean AUC of DOX (1320 ± 260 ng × h/ml) with 30 mg/m² of safingol was not

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**Table 2** Patients characteristics

<table>
<thead>
<tr>
<th>No. of patients entered</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>59 (29–77)</td>
</tr>
<tr>
<td>Median KPS (range)</td>
<td>80 (70–90)</td>
</tr>
<tr>
<td>Male : female</td>
<td>10 : 7</td>
</tr>
<tr>
<td>Tumor types</td>
<td>Pancreatic: 6, Gastric: 2, Colon: 3, Unknown primary: 2, Sarcoma: 3, Nasopharyngeal: 1</td>
</tr>
</tbody>
</table>
from the data. The results indicate that the increase in \( C_{\text{max}} \) is linear with increasing safingol dose. The equations shown represent the best fit regression line studied and plotted against safingol dose (mg/m\(^2\)).

For dose 1 (safingol without DOX), the mean safingol half-life was \( 4.97 \pm 2.51 \) h, the mean estimated clearance was \( 3140 \pm 761 \) nl/min, and the mean volume of distribution was \( 995 \pm 935 \) liters (Fig. 4B). With safingol alone, two patients (nos. 15 and 17) exhibited an increase in their hematological toxicity. Two other patients (nos. 15 and 17) exhibited an increase in their hemoglobin. However, because these changes were not evident when safingol was administered with DOX (Fig. 4B), the basis for the change in the shape of the \( C \times t \) is unclear and awaits further investigation.

### Clinical Response to Safingol and DOX

We have observed signs of clinical activity in several patients. One patient with pancreatic cancer treated at a safingol level of 15 mg/m\(^2\) had a 30% decrease in the size of a measurable omental metastasis (from 41 cm\(^2\) to 26 cm\(^2\)). In addition, she had a decrease in biochemical markers associated with her disease. These included a decrease in CA 19-9 from 3300 units/ml to 2200 units/ml and a decrease in CA-125 from 428 units/ml to 220 units/ml. A second patient with pancreatic cancer at a safingol dose of 15 mg/m\(^2\) experienced a decrease in the size of his pancreatic mass from 41 cm\(^2\) to 26 cm\(^2\). In addition, she had a decrease in the size of a measurable metastasis from 26 cm\(^2\) to 18 cm\(^2\). A third patient with pancreatic cancer treated at a safingol level of 15 mg/m\(^2\) experienced clinical improve-

### Hematological toxicity with safingol and DOX

<table>
<thead>
<tr>
<th>Safingol (mg/m(^2))</th>
<th>DOX (mg/m(^2))</th>
<th>No. of patients</th>
<th>Mean nadir WBC ( \times 10^3 ) (range)</th>
<th>Mean nadir ANC ( \times 10^3 ) (range)</th>
<th>Mean platelet ( \times 10^3 ) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>60</td>
<td>3</td>
<td>0.4 (0.4-0.6)</td>
<td>0.4 (0.1-0.9)</td>
<td>55 (30-99)</td>
</tr>
<tr>
<td>15</td>
<td>45</td>
<td>3</td>
<td>3.29 (1.4-5.0)</td>
<td>1.2 (0.6-1.6)</td>
<td>150 (121-196)</td>
</tr>
<tr>
<td>30</td>
<td>45</td>
<td>4</td>
<td>5.9 (3.1-9.0)</td>
<td>3.0 (2.0-4.0)</td>
<td>236 (150-349)</td>
</tr>
<tr>
<td>60</td>
<td>45</td>
<td>3</td>
<td>4.0 (2.2-6.1)</td>
<td>2.3 (0.7-4.7)</td>
<td>154 (70-233)</td>
</tr>
<tr>
<td>120</td>
<td>45</td>
<td>3</td>
<td>5.4 (4.9-7.5)</td>
<td>4.3 (2.3-5.1)</td>
<td>263 (225-279)</td>
</tr>
</tbody>
</table>

### Effect of safingol on DOX (45 mg/m\(^2\)) AUC

<table>
<thead>
<tr>
<th>Safingol dose (mg/m(^2))</th>
<th>No. of patients</th>
<th>Mean DOX AUC (ng x h/ml, ± SD)</th>
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</thead>
<tbody>
<tr>
<td>15</td>
<td>3</td>
<td>185 ± 94</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>232 ± 78</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
<td>557 ± 61</td>
</tr>
<tr>
<td>120</td>
<td>3</td>
<td>1251 ± 317</td>
</tr>
</tbody>
</table>

### Effect of DOX (45 mg/m\(^2\)) on safingol pharmacokinetics

<table>
<thead>
<tr>
<th>Safingol dose (mg/m(^2))</th>
<th>No. of patients</th>
<th>Mean safingol AUC without DOX (ng x mg/ml, ± SD)</th>
<th>Mean safingol AUC with DOX (ng x mg/ml, ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>3</td>
<td>185 ± 94</td>
<td>151 ± 84</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>232 ± 78</td>
<td>353 ± 260</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
<td>557 ± 61</td>
<td>450 ± 24</td>
</tr>
<tr>
<td>120</td>
<td>3</td>
<td>1251 ± 317</td>
<td>1226 ± 262</td>
</tr>
</tbody>
</table>

### Pharmacokinetic dose-ranging for \( C_{\text{max}} \) (A) and AUC (B) of safingol. \( C_{\text{max}} \) (ng/ml) and AUC (ng x h/ml) are shown for each patient studied and plotted against safingol dose (mg/m\(^2\)). \( r^2 \) = estimate of variability. The equations shown represent the best fit regression line from the data. The results indicate that the increase in \( C_{\text{max}} \) and AUC is linear with increasing safingol dose.
Safingol Alone and in Combination with Doxorubicin

DISCUSSION

Safingol, the L-threo enantiomer of dihydrosphingosine, is the first PKC-specific inhibitor to enter clinical trials. Members of the PKC family are characterized by a unique NH₂-terminal regulatory domain containing cofactor binding sites and a COOH-terminal catalytic domain that is homologous to that of other protein kinases (15, 16). Sphingosines have been shown to inhibit PKC activity and phorbol myristate acetate binding by interfering with the function of the unique regulatory domain of PKC (17). This is in contrast to the “classic” PKC inhibitor, staurosporine, which inhibits PKC activity by interacting with the catalytic domain of the enzyme (18). However, because the catalytic domain of PKC is highly homologous to the catalytic domain of other protein kinases that are critical for normal cellular function (i.e., pp60-src tyrosine kinase and cyclic AMP-dependent protein kinase A) staurosporine has proven exceptionally toxic (19). Safingol, on the other hand, in view of its specificity for the unique regulatory domain of PKC, had been shown in animals not to cause such toxicity, and, therefore, appeared to be an attractive agent for clinical development (20, 21).

The starting dose of safingol was selected as 15 mg/m². This dose represented one-tenth of the mouse LD₅₀, 4% of the HNTD in dogs, and 12% of the HNTD in rats. These HNTDs for dogs and rats did not increase the toxicity of minimally or moderately toxic doses of DOX in these species (11). The study design, though, did allow us to assess the acute toxicity of safingol as a 1-h infusion administered 2 weeks before the combination with DOX. This clinical trial has shown that safingol, up to a dose of 120 mg/m², is well tolerated both by itself and when administered in combination with 45 mg/m² of DOX. There was no dose-limiting hematological toxicity.

From the mice studies with safingol, it was reported that to achieve meaningful chemopotentiation with DOX, it was necessary to deliver a safingol dose of at least 5 mg/kg (9). From the animal studies, a single 5 mg/kg i.v. dose of safingol resulted in a mean plasma Cmax and AUC of 1797 ng/ml and 668 ng X h/ml, respectively (20). In our clinical trial at the highest dose of safingol evaluated (120 mg/m²), the mean Cmax was 988 ng/ml, and the mean AUC was 1277 ng X h/ml. Thus, when compared to the pharmacological data obtained at the 5-mg/kg dose in the rodents, in this Phase I study we were able to approach a therapeutic Cmax for safingol as a chemopotentiating agent for DOX and, in fact, had exceeded the lower theoretical, therapeutic AUC for this drug. More definitive resolution of this pharmacological end point awaits renewal of drug supply.

The hematological toxicity encountered in the three patients treated with 15 mg/m² safingol and 60 mg/m² of DOX was unexpected and was probably related directly to the dose of DOX rather than to an increase in the toxicity of DOX by safingol. In preclinical testing, safingol had no effect when tested as a single agent on colony-forming ability of normal human bone marrow progenitor cells in vitro at concentrations 2–3-fold greater than that required to inhibit tumor cell proliferation by 50%. In addition, when tested in combination, safingol did not potentiate the cytotoxicity of doxorubicin or cisplatin on normal bone marrow progenitor cells (11). Although this study was not designed to study the pharmacokinetics of DOX alone without safingol, it appears that the pharmacokinetics of 45 mg/m² of DOX was not affected by safingol, despite increasing safingol concentrations. In addition, the pharmacokinetics for the 60-mg/m² dose of DOX at this level revealed a mean AUC of 1680 ng X h/ml that did not substantially exceed the AUC associated with identical DOX doses in a published series (13, 22). Therefore, we believe that the myelosuppression observed with 15 and 60 mg/m² of DOX was due to poor bone marrow reserve in a predominantly pretreated patient population with advanced metastatic disease. This hypothesis will eventually require further testing.

Although venous irritation and hemolysis were significant and, in some instances, there were dose-limiting toxicities in the animals, up to a safingol dose of 120 mg/m², both as a single agent and in combination with 45 mg/m² of DOX, no

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3 Unpublished studies from Eli Lilly Research Labs.
dose-limiting hemolysis was seen. Data from the preclinical studies indicated a hemolytic potential to safingol. This was determined not to be related to total safingol dose. It was demonstrated that these effects were mitigated by the use of low safingol concentrations and large-sized, high flow veins. By following guidelines that incorporated these observations, we witnessed only one grade 1 toxicity. This was manifested as a transient fall in serum haptoglobin following infusion through a poor peripheral vein. This, in fact, was prevented with subsequent administration of safingol in the same patient through a central venous catheter. Therefore, the hemolysis induced by safingol in the animal models can be avoided when administered along strict guidelines and, if needed, through a central vein in a more dilute concentration.

We observed no major organ toxicity. Renal toxicity, considered a consequence of intravascular hemolysis, was observed in rats and dogs (21) but was not observed in the clinical trial. At the 120-mg/m² level, one patient received a cumulative DOX dose of 400 mg/m² without change in his left ventricular function, indicating no enhancement of DOX-induced cardiac toxicity by safingol. Another concern was hepatic toxicity. Dogs administered 40 mg/kg of safingol developed clinical pathology indicative of hepatobiliary involvement (21). Pharmacokinetic assessment of these animals at this dose level indicated a mean Cmax of 9.033 ng/ml and a mean AUC of 11,094 ng × h/ml. At the highest dose level of safingol (120 mg/m²) evaluated in this clinical study, the mean Cmax (1041 ng/ml) and mean AUC (1251 ng × h/ml) were considerably below these levels and may account for the absence of observed hepatic toxicity.

This study shows that doses of safingol up to 120 mg/m² can be administered alone or in combination with 45 mg/m² of DOX without significant toxicity. Although signs of clinical activity were observed, in terms of clinical design, it is not possible to definitively state whether this may have been from DOX alone and not from the combination therapy. The levels of safingol achieved in the plasma of patients at the highest dose level of safingol tested approach the micromolar concentrations of safingol that inhibit PKC in vitro and in vivo. Thus, this study suggests that the PKC inhibitor safingol, which inhibits signal transduction pathways and potentiates DOX in vivo, can be safely administered to patients at systemic levels associated with PKC inhibition as well as with chemopotentiation.

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