A Phase I Trial of a Potent P-Glycoprotein Inhibitor, Zosuquidar.3HCl Trihydrochloride (LY335979), Administered Orally in Combination with Doxorubicin in Patients with Advanced Malignancies

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ABSTRACT

Purpose: The purpose of this study was to investigate the safety and tolerability of Zosuquidar.3HCl, a potent inhibitor of P-glycoprotein (Pgp), when administered p.o. alone and in combination with doxorubicin and to determine whether Zosuquidar.3HCl affects doxorubicin pharmacokinetics and inhibits Pgp function in peripheral blood natural killer lymphocytes.

Experimental Design: Patients with advanced nonhematological malignancies were eligible for this Phase I trial. Zosuquidar.3HCl and doxorubicin were administered separately during the first cycle of therapy and then administered concurrently. Zosuquidar.3HCl was administered over 4 days, with doses escalated until the occurrence of dose-limiting toxicity. Subsequently, doxorubicin doses were increased from 45 to 75 mg/m². Zosuquidar.3HCl, characterized by cerebellar dysfunction, hallucinations, and palinopsia. The maximum-tolerated dose for oral Zosuquidar.3HCl administered every 12 h for 4 days is 300 mg/m². Zosuquidar.3HCl did not affect doxorubicin myelosuppression or pharmacokinetics, and Zosuquidar.3HCl pharmacokinetics were similar in the absence and presence of doxorubicin. Higher plasma concentrations of Zosuquidar.3HCl were associated with greater Pgp inhibition in natural killer cells.

Conclusion: Zosuquidar.3HCl can be coadministered with doxorubicin using a 4-day oral dosing schedule, with little effect on doxorubicin toxicity or pharmacokinetics. Further refinement in Zosuquidar.3HCl dosing and scheduling should be explored to optimize Pgp inhibition while minimizing cerebellar toxicity.

INTRODUCTION

The effectiveness of natural product-derived anticancer drugs is limited by the ability of many cancer cells to display an intrinsic or acquired multidrug resistance phenotype. Multidrug resistance is often associated with expression of transmembrane efflux proteins resulting in a decreased drug accumulation (1). Among the family of ATP-binding cassette proteins, the M₁₇₀,₀₀₀ Pgp is the most extensively characterized. Expression of this protein is sufficient to confer multidrug resistance in cell culture and in animal models of human cancers (2). Attempts to improve anticancer therapy by coadministration of Pgp inhibitors to date have been disappointing (3). However, many of the earliest Pgp inhibitors were compounds developed for other clinical uses and lacked sufficient potency and/or specificity to adequately test a clinical hypothesis. Many exhibited non-target-related toxicities that compromised the achievement of therapeutic exposures (4).

Zosuquidar.3HCl (LY335979), a difluorocyclopropyl quinoline, was developed specifically as a selective Pgp inhibitor (Fig. 1; Ref. 5). Concentrations of Zosuquidar.3HCl from 50 to 100 nm are effective in modulating Pgp-mediated drug resistance in a variety of cell culture models (6, 7). Zosuquidar.3HCl is also effective in murine syngeneic and xenograft models of Pgp-mediated drug resistance (6). Furthermore, in contrast to other Pgp inhibitors, in animal studies Zosuquidar.3HCl does not alter the pharmacokinetic profile of coadministered Pgp substrates such as doxorubicin, paclitaxel, or etoposide (6).
When taken together, these characteristics indicate that Zosuquidar.3HCl warrants investigation in reversing clinical drug resistance mediated by Pgp. Therefore, we initiated a Phase I trial designed to determine whether biologically effective plasma concentrations of Zosuquidar.3HCl could be achieved in cancer patients who received the drug p.o. The study was also designed to evaluate the effects of Zosuquidar.3HCl on doxorubicin pharmacokinetics and toxicity. The results indicate that although reversible ataxia limits dose escalation of oral Zosuquidar.3HCl, biologically effective plasma concentrations are achievable at doses associated with minimal toxicity and without significant alteration of doxorubicin pharmacokinetics or doxorubicin-induced myelosuppression.

PATIENTS AND METHODS

Patient Selection. Patients who were at least 18 years of age and met all of the following criteria were eligible for the study: (a) histological or cytologic diagnosis of metastatic or locally advanced cancer (must have failed conventional therapy, have disease considered refractory to standard chemotherapy regimens, or have disease for which no standard chemotherapy was available); (b) prior radiation therapy and chemotherapy completed at least 3 weeks before study enrollment (6 weeks if prior treatment was nitrosourea or mitomycin C); (c) lifetime cumulative anthracycline dose must have not exceeded the doxorubicin equivalent of 300 mg/m²; (d) resting blood pool scan with a cardiac ejection fraction of >45%; (e) performance status of 0–2 on the Eastern Cooperative Oncology Group scale; (f) estimated life expectancy of at least 16 weeks; and (g) adequate organ function (granulocytes ≥ 1.5 × 10⁹ cells/liter; platelets ≥ 100 × 10⁹ cells/liter; hemoglobin ≥ 9 g/liter; bilirubin ≤ upper limit of normal; alanine transaminase and aspartate transaminase ≤ 2.5 times normal; serum creatinine ≤ 1.5 mg/dl). Females were required to have child-bearing potential terminated by surgery, radiation, or menopause or attenuated by uses of contraceptives during and for 3 months after the trial. Written informed consent was obtained from all patients according to institutional, state, and federal guidelines.

Treatment and Clinical Evaluation. Patients were entered in the study in cohorts of three and received treatment during multiple cycles of either 35 days (cycle 1) or 21 days (cycle 2 and subsequent cycles; Table 1). The initial Zosuquidar.3HCl dose was based on dog toxicity studies. A 2-week i.v. infusion in dogs demonstrated that a dose of 10 mg/kg/day was a no effect dose (19). Peak plasma concentrations in that study exceeded 1000 nM without demonstrable toxicity. The initial human dose (20 mg/m²/day) was chosen to be more than 10-fold lower than the equivalent no effect dose in the dog. Oral administration every 12 h was initially chosen to balance the anticipated half-life of Zosuquidar.3HCl in man (18 h), the duration of effect on Pgp, and the known pharmacokinetic parameters of doxorubicin in man.

Dose escalation of both Zosuquidar.3HCl and doxorubicin was performed as listed in Table 1 and based on assessment of toxicity. Evaluations performed during treatment included weekly physical examinations, complete blood counts, and serum biochemistry analyses. Cardiac function was assessed by gated blood pool scans performed after every other cycle. Dose-limiting toxicity for Zosuquidar.3HCl alone (determined during cycle 1) or Zosuquidar.3HCl given in combination with doxorubicin (determined during cycle 2) was defined as grade 3 or higher nonhematological toxicity (according to National Cancer Institute Common Toxicity Criteria version 1, excluding alopecia, nausea, or vomiting) or grade 4 hematological toxicity lasting for 5 days or longer. If dose-limiting toxicity occurred in one of the first three patients treated at a given dose level, three additional patients were to be treated at that dose level. If none of these additional patients experienced dose-limiting toxicity, dose escalation was to continue. If dose-limiting toxicity occurred in two of six patients, dose escalation was to stop. The MTD was defined as one dose level lower than the dose level at which at least two of six patients experienced dose-limiting toxicity. A target Zosuquidar.3HCl plasma AUC₂₄ h was also included in the dose-escalation strategy. Dose escalation was to cease if, in a given cohort, plasma Zosuquidar.3HCl AUC₂₄ h was ≥20 µg.h/ml/24 h in all three patients during cycle 1. At least 10 patients were to be treated at the MTD or AUC-targeted dose.

Zosuquidar.3HCl was administered p.o. on days 1 through 4 every 12 h for 7 doses (cohorts 1–5 and cohort 9B) or, based on early pharmacokinetic data, every 8 h for 10 doses (cohorts 6, 7, 8, and 9A; Fig. 2). Doxorubicin was administered i.v. over 30 min on either day 15 (cycle 1 only) or day 3 (cycle 2 and subsequent cycles). For day 3 administrations, doxorubicin was administered 2 h after the morning dose of Zosuquidar.3HCl. This dosing schedule was developed to allow steady-state plasma levels to be achieved before the administration of doxorubicin. Doxorubicin doses were adjusted for neutrophil and platelet nadirs occurring during the preceding course of therapy, according to the following algorithm: (a) no change for neutrophil nadir ≥ 0.5 × 10⁹ cells/liter and platelets ≥ 50 × 10⁹ cells/liter; (b) 25% dose reduction for neutrophil nadir < 0.5 × 10⁹ cells/liter and platelets ≥ 50 × 10⁹ cells/liter; (c) 50% dose reduction for neutrophil nadir < 0.5 × 10⁹ cells/liter and platelets < 50 × 10⁹ cells/liter but ≥ 25 × 10⁹ cells/liter; and (d) 75% dose reduction for neutrophil nadir < 0.5 × 10⁹ cells/liter and platelets < 50 × 10⁹ cells/liter but ≥ 25 × 10⁹ cells/liter, or if platelets were < 25 × 10⁹ cells/liter.

Antitumor response was evaluated after the third cycle of therapy and after every other subsequent cycle. Responses were quantified by either physical examination or appropriate imaging studies according to WHO criteria (9).
Phase I Trial of Oral Zosuquidar

Potassium phosphate and acetonitrile (72:28, v:v) in phosphoric acid (pH 3.0), and a flow rate of 1.0 ml/min. A Shimadzu RF-551 spectrophotometric detector was used (λex , 470 nm; λem , 550 nm) to quantify doxorubicin and doxorubicinol.

Pharmacokinetic Analysis. Plasma pharmacokinetic parameters of Zosuquidar.3HCl, doxorubicin, and doxorubicinol were evaluated using noncompartmental methods (WinNonlin Professional version 2.1). Plasma AUC, CL, volume of distribution at steady state (Vss), and terminal half-life (t1/2) were calculated for doxorubicin and plasma AUC and Cmax (observed maximum plasma concentration) for doxorubicinol as follows:

$$AUC = \frac{D}{CL} + \frac{C(t_n)^+}{\lambda_z}$$ (1)

where t_n is the last time point where the plasma concentration is above the limit of quantitation, C(t_n)^+ is the prediction for the concentration at the last quantifiable time point, and λ_z is the calculated terminal rate constant;

$$CL = \frac{D}{AUC}, \text{ where } D = \text{ dose}$$ (2)

$$Vss = CL \times MRT_{\text{ss}}, \text{ where}$$ (3)

$$MRT_{\text{ss}} = \frac{AUMC}{AUC} - \frac{T}{2}$$ (4)

with T the infusion time and

$$AUMC = AUMC(0 - t_0) + \frac{C(t_n)^+}{\lambda_z} + \frac{C(t_n)^+}{\lambda_z^2}$$ (5)

where AUMC is area under the moment curve.

For Zosuquidar.3HCl, apparent oral CL at day 4 (CL/Fday 4) was calculated as D/AUC_{day 4} where AUC_{day 4} = AUC for the dosing interval at day 4. Apparent volume of distribution at day 4 (Vss/Fday 4) was determined as CL/Fday 4 divided by λ_z. Maximum drug concentrations in plasma on day 4 (C_{max day 4}) and minimum concentrations on day 4 (C_{min day 4}) were determined directly from the observed concentration-time data.

Summary statistics were calculated for cycle 1 and 2 pharmacokinetic parameters, which were compared using a t test. Logistic regression was used to analyze relationships between

\[
\begin{array}{|c|c|c|c|c|c|}
\hline
\text{Cohort} & \text{No. of} & \text{Zosuquidar.3HCl} & \text{Total no.} & \text{Time between} & \text{Doxorubicin} \\
\text{no.} & \text{patients} & \text{dose (mg/m}^2\text{)} & \text{doses} & \text{doses (h)} & \text{dose (mg/m}^2\text{)} \\
\hline
1 & 3 & 20 & 7 & 12 & 45 \\
2 & 4 & 40 & 7 & 12 & 45 \\
3 & 3 & 80 & 7 & 12 & 45 \\
4 & 4 & 160 & 7 & 12 & 45 \\
5 & 3 & 320 & 7 & 12 & 45 \\
6 & 3 & 400 & 10 & 8 & 45 \\
7 & 3 & 300 & 10 & 8 & 45 \\
8a & 3 & 300 & 10 & 8 & 60 \\
9A & 2 & 300 & 10 & 8 & 75 \\
9B & 10 & 300 & 7 & 12 & 75 \\
\hline
\end{array}
\]

\(*a\) Zosuquidar.3HCl was administered as a capsule beginning with this cohort.

Fig. 2 Schedule of administration of Zosuquidar.3HCl and doxorubicin. Zosuquidar.3HCl was administered p.o. either every 12 h for 7 doses (cohorts 1–5 and 9B) or every 8 h for 10 doses (cohorts 7, 8, and 9A). For cycles 2 and higher (2+), doxorubicin was administered i.v. on day 3, 2 h after the morning dose of Zosuquidar.3HCl.

Plasma Drug Quantitation. Plasma samples were obtained to determine concentrations of Zosuquidar.3HCl, doxorubicin, and the metabolite doxorubicinol when the drugs were administered separately (cycle 1) and in combination (cycle 2). Plasma sampling for Zosuquidar.3HCl occurred predose and at 0.5, 1, 1.5, 2, 4, 6, 9, 24, 31, 46, 70, and 96 h after doxorubicin dose.

Zosuquidar.3HCl concentrations were analyzed using a validated (20–2000 ng/ml) reverse-phase HPLC assay. After addition of an internal standard, plasma samples were subjected to solid phase extraction using a styrene divinylbenzene (LMS; Varian) extraction cartridge. HPLC was performed using a Zorbax RX-C8 column (4.6 × 150 mm), an isocratic mobile phase consisting of acetonitrile and 35 mm ammonium acetate [pH 4.8 (70:30, v:v)], and a flow rate of 1.0 ml/min. Zosuquidar.3HCl was quantitated using a Hitachi L 7480 fluorescence detector (λex , 240 nm; λem , 415 nm).

Plasma samples were analyzed for doxorubicin and doxorubicinol using a validated HPLC method. After addition of an internal standard (epirubicin), the samples were subjected to solid phase extraction using a C-18 extraction cartridge (Varian). HPLC was performed using a Zorbax SB-C8 column (4.6 × 25 cm), an isocratic mobile phase consisting of 20 mM potassium phosphate and acetonitrile (72:28, v:v) in phosphoric acid (pH 3.0), and a flow rate of 1.0 ml/min. A Shimadzu RF-551 spectrophotometric detector was used (λex , 470 nm; λem , 550 nm) to quantify doxorubicin and doxorubicinol.

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$$AUC = \frac{D}{CL} + \frac{C(t_n)^+}{\lambda_z}$$ (1)

where t_n is the last time point where the plasma concentration is above the limit of quantitation, C(t_n)^+ is the prediction for the concentration at the last quantifiable time point, and λ_z is the calculated terminal rate constant;

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Summary statistics were calculated for cycle 1 and 2 pharmacokinetic parameters, which were compared using a t test. Logistic regression was used to analyze relationships between
Evaluation of Pgp Function in Peripheral Blood Cells. A surrogate assay of Pgp function in patients was used, using peripheral blood natural killer (CD56+ lymphocytes, a subset known to express Pgp (7, 10, 11). Briefly, mononuclear cells were collected at 0 (presample), 72, 73, and 96 (postsamples) h after the start of Zosuquidar.3HCl dosing from patients using Becton Dickinson CPT tubes. The cells were separated by centrifugation (1500 × g for 20 min), washed, and resuspended in serum-free RPMI 1640 at a concentration of 1 × 10^6 cells/ml. At each time point, the sample was divided into two aliquots. Zosuquidar.3HCl (100 nm final concentration) was added to one of these aliquots (spike sample) to determine the maximum possible Pgp inhibition, and RPMI 1640 buffer was added to the other aliquot as a control (buffer sample). The cells were incubated with 50 ng/ml rhodamine 123 at 37°C for 90 min. Subsequently, 20 μL of CD56 Cy-Chrome antibody (PharMingen) were added, and the cells were incubated for an additional 15 min at 37°C. The samples were analyzed using an Epics XL flow cytometer and dual fluorescence quantitation. Ten thousand cells were analyzed for green (rhodamine) and red (CD56+) fluorescence using 525 nm bandpass and 630 nm long pass filters, respectively. The MFI for each sample was determined in duplicate. The results are expressed as percentage inhibition of rhodamine 123 efflux relative to the Zosuquidar.3HCl spike sample using the formula below:

\[
\text{% inhibition} = 100 \times \frac{[\text{MFI}_\text{pre(buffer)} - [\text{MFI}_\text{pre(buffer)} \times \text{MFI}_\text{post(spike)}]/[\text{MFI}_\text{pre(spike)}]}{[\text{MFI}_\text{pre(spike)} - [\text{MFI}_\text{pre(buffer)} \times \text{MFI}_\text{post(spike)}]/[\text{MFI}_\text{pre(buffer)}]}
\]

RESULTS

Patient Characteristics. Selected characteristics of the 38 patients enrolled in the trial are listed in Table 2. The patients had a variety of tumor types, and most common tumor type was breast cancer. Metastatic disease was present in 36 patients, and

Zosuquidar.3HCl pharmacokinetic parameters and occurrence of toxicity.

Grade 3/4 cerebellar toxicity associated with Zosuquidar.3HCl administration

<table>
<thead>
<tr>
<th>Zosuquidar.3HCl dose (mg/m²)</th>
<th>No. of patients</th>
<th>Cycle 1</th>
<th>Cycle ≥2</th>
</tr>
</thead>
<tbody>
<tr>
<td>320 q12h</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>400 q8h</td>
<td>3</td>
<td>3</td>
<td>0*</td>
</tr>
<tr>
<td>300 q12h</td>
<td>8</td>
<td>3</td>
<td>1*</td>
</tr>
<tr>
<td>300 q12h</td>
<td>10</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Only one patient received cycle 2 therapy in this cohort; for this patient cycle 2 included a dose reduction of Zosuquidar.3HCl to 300 mg/m².

Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with cerebellar toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female—20 Male—18</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>Mean—56 Range—33–78</td>
</tr>
<tr>
<td>ECOG status</td>
<td>0–14 1–21 2–3</td>
</tr>
<tr>
<td>Tumor type</td>
<td>Breast—11 NSC lung—5 Kidney—4</td>
</tr>
<tr>
<td>Prior chemotherapy</td>
<td>36</td>
</tr>
<tr>
<td>Prior doxorubicin</td>
<td>8</td>
</tr>
<tr>
<td>Prior radiotherapy</td>
<td>23</td>
</tr>
</tbody>
</table>

a ECOG, Eastern Cooperative Oncology Group; NSC, non-small cell.
higher). Grade 4 neutropenia occurred in cycle 1 or 2 in only two patients and did not last more than 5 days in any patient. One of these patients was enrolled in cohort 8 (300 mg/m² Zosuquidar.3HCl q8h and 60 mg/m² doxorubicin) and experienced grade 4 neutropenia in cycle 1 only. The other patient was treated in cohort 9B (300 mg/m² Zosuquidar.3HCl q12h and 75 mg/m² doxorubicin) and experienced grade 4 neutropenia in both cycle 1 and 2 (despite a 25% reduction in doxorubicin dose for cycle 2). Overall, the cycle 2 doxorubicin dose was decreased due to cycle 1 neutropenia in nine patients. Analysis of mean nadir counts for granulocytes and platelets during cycles 1 and 2 suggests that concurrent oral administration of Zosuquidar.3HCl does not alter the hematological toxicity associated with doxorubicin (Table 4).

Significant nonhematological toxicities associated with doxorubicin or the combination of doxorubicin and Zosuquidar.3HCl were also uncommon. Three patients had a >10% decrease in cardiac left ventricular ejection during or after protocol treatment. One of these patients had breast cancer and had not received doxorubicin previously. After six cycles of treatment with 80 mg/m² Zosuquidar.3HCl q12h and 45 mg/m² doxorubicin (cumulative doxorubicin dose, 225 mg/m²), there was an asymptomatic decrease in cardiac ejection fraction from 56% to 42%. In a second patient with non-small cell lung cancer (who had not been treated with doxorubicin previously), there was an asymptomatic decline in cardiac ejection fraction from 71% to 55% after 2 cycles of treatment with 300 mg/m² Zosuquidar.3HCl q12h and 75 mg/m² doxorubicin (cumulative doxorubicin dose, 107 mg/m²).

A third patient with metastatic breast cancer had previously received 220 mg/m² doxorubicin and had a cardiac ejection fraction of 57% at study entry. Protocol treatment was discontinued after 6 cycles of treatment with 300 mg/m² Zosuquidar.3HCl q12h and 75 mg/m² doxorubicin due to achievement of a cumulative lifetime doxorubicin dose of 540 mg/m². Her cardiac ejection function was unchanged on bi-monthly gated blood pool scans until about 1 month after discontinuation of therapy, when she developed dyspnea. At that time, her ejection fraction was found to be 33%, with cardiac ultrasound indicating four chamber enlargement. Her symptoms improved with diuretics and angiotensin-converting enzyme inhibitors. A gated blood pool scan done 3 months after discontinuation of therapy indicated that her ejection fraction was 22%, and her cardiorespiratory symptoms were stable at that time.

**Antitumor Responses.** Among 24 patients who were evaluable for antitumor responses, 1 patient had a partial response to therapy. This patient had recurrent and metastatic breast cancer, manifested by left breast and axillary masses. She had previously received adjuvant cyclophosphamide, methotrexate, and 5-fluorouracil, as well as paclitaxel, cis-retinoic acid, and IFN for metastatic disease. After cycle 2 of Zosuquidar.3HCl/doxorubicin, there was a >50% decrease in the breast mass, and the axillary mass was not detectable. The patient subsequently received a total of 7 cycles of therapy, with treatment discontinued due to achievement of a cumulative doxorubicin dose of >500 mg/m².

Seven patients had stable disease as their best response to treatment, including five patients who received ≥6 cycles (5 months) of therapy. A patient with renal cell carcinoma, for whom therapy was discontinued due to achievement of a maximum cumulative doxorubicin dose, remained free from disease progression for approximately 1 year after treatment discontinuation.

**Pharmacokinetics and Pharmacodynamics.** The geometric mean and CV of selected doxorubicin and doxorubicinol pharmacokinetic parameters for cycles 1 and 2 are listed in Tables 5 and 6. Doxorubicin CL, AUC, and apparent volume of distribution were similar in the presence or in absence of Zosuquidar.3HCl for doxorubicin doses of 45 or 75 mg/m² (Table 5). In addition, the terminal elimination half-life observed for doxorubicin remained unchanged after multiple oral doses of doxorubicin and is consistent with values reported in the literature (12). Doxorubicinol AUC and $C_{\text{max}}$ were also similar in the presence or absence of Zosuquidar.3HCl (Table 6). Statistical analyses indicated that there were no significant differences between cycle 1 and 2 pharmacokinetic parameters for doxorubicin and doxorubicinol, suggesting that Zosuquidar.3HCl has little, if any, effect on doxorubicin and doxorubicinol pharmacokinetics.

With regard to Zosuquidar.3HCl pharmacokinetics, cycle 1 day 4 $AUC_{\text{day 4}}$, $C_{\text{max}}$ day 4, and $C_{\text{min}}$ day 4 tended to increase as the oral dose increased (Table 7). The relatively large interpatient variability in pharmacokinetic parameters and the rather narrow dose range precluded an analysis of linearity with respect to dose. There was no correlation between day 4

### Table 4  Mean granulocyte and platelet nadir counts by cycle and cohort

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Cycle 1 (range)</th>
<th>Cycle 2 (range)</th>
<th>Platelets ($\times 10^9$/liter)</th>
<th>Cycle 1 (range)</th>
<th>Cycle 2 (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.7 (1.2–2.3)</td>
<td>2.6 (1.3–3.5)</td>
<td>252 (98–358)</td>
<td>306 (186–476)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.9 (2.4–14.0)</td>
<td>3.9 (1.3–5.4)</td>
<td>269 (106–430)</td>
<td>225 (132–348)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.8 (2.1–6.1)</td>
<td>2.8 (1.9–3.6)</td>
<td>198 (171–251)</td>
<td>208 (162–253)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3.8 (2.3–5.6)</td>
<td>4.1 (3.8–4.6)</td>
<td>309 (189–427)</td>
<td>312 (147–429)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9.7 (2.3–13.5)</td>
<td>6.0 (1.1–10.9)</td>
<td>248 (153–324)</td>
<td>168 (136–199)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.0 (1.0–1.0)</td>
<td>2.0 (2.0–2.0)</td>
<td>170 (170–170)</td>
<td>162 (162–162)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.7 (1.8–3.3)</td>
<td>3.3 (1.2–7.0)</td>
<td>120 (103–142)</td>
<td>154 (45–250)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.7 (0.9–8.0)</td>
<td>5.3 (1.4–13.1)</td>
<td>214 (57–423)</td>
<td>341 (129–743)</td>
<td></td>
</tr>
<tr>
<td>9A</td>
<td>1.8 (1.8–1.8)</td>
<td>1.2 (1.2–1.2)</td>
<td>161 (161–161)</td>
<td>142 (142–142)</td>
<td></td>
</tr>
<tr>
<td>9B</td>
<td>1.8 (0.7–2.5)</td>
<td>1.8 (0.8–3.9)</td>
<td>190 (102–294)</td>
<td>150 (48–351)</td>
<td></td>
</tr>
</tbody>
</table>
Zosuquidar.3HCl AUC, \( C_{\text{max}} \), or \( C_{\text{min}} \) and the occurrence of cerebellar toxicity.

To facilitate comparison of cycle 1 and 2 Zosuquidar.3HCl pharmacokinetic parameters, data were pooled for 11 patients for whom complete pharmacokinetic data were available for both cycles 1 and 2 (Table 8). The terminal half-life of Zosuquidar.3HCl was similar for cycles 1 and 2. By contrast, apparent oral CL and apparent volume of distribution increased from cycle 1 to cycle 2 (47% and 30%, respectively; Table 8). However, these results should be interpreted with caution because statistical analyses indicated that the increase in apparent oral CL was of borderline significance (\( P = 0.051 \)), whereas the difference in apparent volume of distribution was not significant (\( P = 0.1 \)).

The effects of Zosuquidar.3HCl administration on Pgp function were studied in peripheral blood mononuclear cells obtained at specified times after drug administration. Natural killer cells are known to express Pgp and can be identified by their expression of CD56 (11). Assessment of Pgp function was analyzed in these cells by incubation in rhodamine dye, with increased rhodamine retention reflecting Pgp inhibition. The results indicate that higher concentrations of plasma Zosuquidar.3HCl were associated with greater Pgp inhibition (Fig. 3).

**DISCUSSION**

Recently, second- or third-generation MDR-modulating agents have entered clinical trials. These agents, such as valsipodar (PSC-833) and biricodar (VX-710), are characterized by enhanced potency against Pgp with fewer non-target-related toxicities. Valsipodar is perhaps the most extensively studied Pgp modulator in the clinic to date. With administration of valsipodar, plasma concentrations capable of inhibiting Pgp in vivo can be attained consistently (13, 14). However, significant alterations in the CL of coadministered chemotherapeutic agents with consequent increases in overall exposure required dose reductions of the cytotoxic agent administered concurrently with valsipodar. In a study of valsipodar administered with doxorubicin, valsipodar administration resulted in an approximately 30% decrease in doxorubicin CL, significant increases in doxorubicin AUC, and dramatic increases in the AUC of doxorubicinol (15). The extent to which the pharmacokinetic interactions observed are related to Pgp interactions versus inhibition of other ATP-

---

### Table 5 Pharmacokinetic parameters for doxorubicin (Dox) in the absence (cycle 1) and presence (cycle 2) of oral Zosuquidar.3HCl

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cycle 1 (n = 20)</th>
<th>Cycle 2 (n = 13)</th>
<th>Cycle 1 (n = 8)</th>
<th>Cycle 2 (n = 3)</th>
<th>Cycle 1 (n = 31)</th>
<th>Cycle 2 (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (( \mu g/h/liter ))</td>
<td>1423 (23.6)</td>
<td>1467 (23.3)</td>
<td>2495 (19.1)</td>
<td>2417 (8.97)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CL (liter/h)</td>
<td>59.1 (23.0)</td>
<td>56.6 (22.9)</td>
<td>55.5 (23.8)</td>
<td>56.8 (18.0)</td>
<td>58.0 (23.0)</td>
<td>53.5 (23.9)</td>
</tr>
<tr>
<td>Vss (liter)</td>
<td>2197 (32.3)</td>
<td>2260 (30.9)</td>
<td>2425 (28.2)</td>
<td>2484 (13.0)</td>
<td>2297 (31.2)</td>
<td>2193 (27.2)</td>
</tr>
<tr>
<td>t (h)</td>
<td>39.6 (27.5)</td>
<td>39.6 (21.5)</td>
<td>47.7 (9.51)</td>
<td>41.6 (6.05)</td>
<td>41.6 (23.8)</td>
<td>39.8 (16.6)</td>
</tr>
</tbody>
</table>

---

### Table 6 Pharmacokinetic parameters for doxorubicin (Dox) in the absence (cycle 1) and presence (cycle 2) of oral Zosuquidar.3HCl

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dox dosea 45 mg/m²</th>
<th>Combined Dox dosesa 45-60-75 mg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (( g/h/liter ))</td>
<td>11.6 (50.9)</td>
<td>12.1 (38.6)</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (( \mu g/liter ))</td>
<td>300 q8h 7 and 8</td>
<td>139.7 (54.4)</td>
</tr>
<tr>
<td>( C_{\text{min}} ) (( g/liter ))</td>
<td>45 mg/m² Dox dose</td>
<td>235 (20)</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>139.7 (54.4)</td>
<td>182.4 (154.6)</td>
</tr>
</tbody>
</table>

---

### Table 7 Pharmacokinetic parameters for Zosuquidar.3HCl by dose level (cycle 1)

<table>
<thead>
<tr>
<th>Dosea (mg/m²)</th>
<th>Cohort (no. of patients)</th>
<th>AUC(0-( t_{1/2} )) (( \mu g/h/liter ))</th>
<th>( C_{\text{max}} ), day 4 (( \mu g/liter ))</th>
<th>( C_{\text{min}} ), day 4 (( \mu g/liter ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>160 q12h</td>
<td>4 (n = 3)</td>
<td>647 (509–772)</td>
<td>132.6 (126.6–139.7)</td>
<td>26 (21.5–29)</td>
</tr>
<tr>
<td>300 q12h</td>
<td>9B (n = 5)</td>
<td>1407 (627–2469)</td>
<td>264.5 (93.1–435.7)</td>
<td>66.4 (33.3–105.6)</td>
</tr>
<tr>
<td>320 q12h</td>
<td>5 (n = 3)</td>
<td>1149 (822–1425)</td>
<td>182.4 (154.6–215.3)</td>
<td>43.8 (31.4–54.4)</td>
</tr>
<tr>
<td>300 q8h</td>
<td>7 and 8 (n = 5)</td>
<td>939 (526–2970)</td>
<td>178.6 (94.1–522.6)</td>
<td>67.1 (32.1–235)</td>
</tr>
<tr>
<td>400 q8h</td>
<td>6 (n = 1)</td>
<td>1396</td>
<td>332.3</td>
<td>74.2</td>
</tr>
</tbody>
</table>

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# References

1. Zosuquidar.3HCl dose 20 to 400 mg/m².
2. Zosuquidar.3HCl dose 300 mg/m².
3. N/A, not applicable.

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Data are expressed as geometric mean (range).
Phase I Trial of Oral Zosuquidar + Doxorubicin

binding drug transporters (such as MRP-2) or metabolic pathways (such as CYP3A) is not fully understood. Randomized clinical trials in which valspodar was combined with dose-reduced cytotoxic agents have yielded disappointing results to date (16, 17).

Zosuquidar.3HCl is a potent new Pgp modulator that does not inhibit other members of the ATP-binding drug transporter family (such as MRP-1, MRP-2, or BCRP) or affect P450 isozymes at concentrations below the micromolar range (18). Pharmacokinetic interactions with anthracyclines were not detectable in preclinical studies performed in dogs (8). The results obtained in the present study are largely consistent with these preclinical data, indicating that at doses associated with Pgp inhibition, Zosuquidar.3HCl does not markedly alter doxorubicin elimination or exposure. Specifically, no significant differences in doxorubicin AUC, CL, volume of distribution, or half-life were observed between cycle 1 (absence of Zosuquidar.3HCl) and cycle 2 (presence of Zosuquidar.3HCl; Table 5). Likewise, little effect was noted on the elimination of the metabolite, doxorubicinol (Table 6). A possible explanation for the differing effects of Zosuquidar.3HCl on doxorubicin pharmacology as compared with other Pgp-targeting compounds is that Zosuquidar.3HCl should not alter non-Pgp-mediated hepatic CL of doxorubicin, which may be mediated by MRP2/c-MOAT (19). MRP-2 is a conjugate export pump located on the hepatocyte canalicular membrane and is capable of transporting bilirubin conjugates (20). The simultaneous inhibition of both MRP-2 and Pgp by compounds less specific than Zosuquidar.3HCl may eliminate the two major export pathways of doxorubicin, resulting in significant alterations in the overall elimination of the drug. Notably, administration of Zosuquidar.3HCl is not associated with transient hyperbilirubinemia, which was reported for valspodar and may relate to inhibition of both MRP2 and Pgp by this drug (14).

Within the context of this dose-escalation trial, the surrogate assay using peripheral blood cells showed complete inhibition of Pgp by Zosuquidar.3HCl in relatively few patients. Thus, we cannot exclude the possibility that at Zosuquidar.3HCl exposures that result in complete Pgp inhibition in a higher percentage of patients, effects on doxorubicin and/or doxorubicinol pharmacokinetics may be observed. Nevertheless, when individual patient data are examined, no significant differences in doxorubicin pharmacology are evident between those patients that received higher (cohorts 5–9) versus lower (cohorts 1–4) doses of Zosuquidar.3HCl (data not shown).

This trial was also designed to assess whether Zosuquidar.3HCl enhanced the known toxicities of doxorubicin. No significant differences were observed in doxorubicin-induced nadir leukocyte and platelet counts in the presence and absence of Zosuquidar.3HCl (Table 4). In addition, patients were monitored closely for cardiac function throughout the course of the study. Two patients discontinued the clinical trial due to a >10% fall in left ventricular ejection fraction, but both were asymptomatic and remained without evidence of congestive heart failure. Although a third patient developed congestive heart failure 1 month after discontinuation of therapy, this patient had received a cumulative dose of doxorubicin of greater than 500 mg/m², with this dose associated with an approximately 7% risk of heart failure (21).

Administration of other Pgp inhibitors has been associated with ataxia (14), which proved to be the dose-limiting toxicity for oral administration of Zosuquidar.3HCl. This finding is consistent with a Pgp target-related effect, perhaps due to disruption of the blood-brain barrier (22). However, there are important differences in the ataxia described for valspodar and that observed with Zosuquidar.3HCl. With the former, the ataxia occurs within a short time after the administration of the first dose and is noted with both oral and i.v. formulations (14, 23). With Zosuquidar.3HCl, ataxia became apparent only after 24 h or more of dosing and was not observed when Zosuquidar.3HCl was administered i.v., despite achieving similar plasma concentrations relative to this study. The lack of dose-limiting ataxia in the i.v. study suggests that inhibition of Pgp within the blood-brain barrier is not sufficient to explain the ataxia observed with oral Zosuquidar.3HCl administration. In this regard,

Table 8 Pharmacokinetic parameters of Zosuquidar.3HCl after multiple oral doses in the absence (cycle 1) or presence (cycle 2) of doxorubicin (n = 11)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/Fday[^a] (liter)</td>
<td>421 (63.4) (n = 11)</td>
<td>617 (37.5) (n = 11)</td>
<td>+47</td>
</tr>
<tr>
<td>V/F[^a] (liter)</td>
<td>17425 (68.3) (n = 7)</td>
<td>22718 (71.7) (n = 7)</td>
<td>+30</td>
</tr>
<tr>
<td>t1/2[^a] (h)</td>
<td>24.4 (119.4) (n = 7)</td>
<td>24.5 (50.3) (n = 7)</td>
<td>+0.7</td>
</tr>
</tbody>
</table>

[^a] Zosuquidar.3HCl dose 20 to 400 mg/m² every 8 or 12 h.
[^b] Apparent oral CL on day 4.
[^c] Apparent volume of distribution on day 4.
a study in which Zosuquidar.HCl was incubated with human hepatic microsomes demonstrated that Zosuquidar.HCl is rapidly and extensively metabolized by the liver (24). Thus, the formation of a first-pass metabolite in the setting of Pgp inhibition of the blood-brain barrier may contribute to the ataxia observed with oral Zosuquidar.HCl administration.

In summary, this study demonstrated that using a 4-day dosing schedule and oral administration, Zosuquidar.HCl can be administered safely in combination with doxorubicin. The 4-day dosing schedule was associated with dose-limiting neurocerebellar toxicity and Zosuquidar.HCl plasma levels that did not result in complete Pgp inhibition in all of the patients, although patients at the highest doses had the highest percentage of target inhibition. The possibility of metabolite-mediated cerellar toxicity suggests that alternative dosing schedules that yield more complete Pgp inhibition with less toxicity should be explored. Although the half-life of Zosuquidar is about 24 h, the drug is extensively distributed, and plasma levels decline rapidly during the distribution phase. Administering two doses 12 h apart on a single day may allow higher plasma concentrations to be achieved while reducing metabolite-mediated toxicity. The results of this study support the continued investigation of the combination of oral Zosuquidar.HCl with doxorubicin and other Pgp substrates.

REFERENCES


A Phase I Trial of a Potent P-Glycoprotein Inhibitor, Zosuquidar.3HCl Trihydrochloride (LY335979), Administered Orally in Combination with Doxorubicin in Patients with Advanced Malignancies

Eric H. Rubin, Dinesh P. de Alwis, Isabelle Pouliquen, et al.


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