Effect of Multidrug Resistance Modulators on the Hepatobiliary Disposition of Doxorubicin in the Isolated Perfused Rat Liver

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ABSTRACT

P-Glycoprotein (P-gp)-mediated multidrug resistance (MDR) in cancer cells may be modulated by competitive inhibitors of P-gp. In the liver, P-gp is localized on the canalicular membrane of hepatocytes. Quinidine and GF120918 inhibit the transport of P-gp substrates, including doxorubicin. Competitive inhibition of P-gp transport may alter biliary excretion of substrates. This study was designed to examine the effects of MDR modulators on the hepatobiliary disposition of doxorubicin and to elucidate the site(s) of drug-modulator interaction using pharmacokinetic modeling techniques. Livers from male Sprague Dawley rats were isolated and perfused for 2 h at 37°C with recirculating male rat blood. MDR modulator (16.8-480 μg of GF120918 or 0.3-3.0 mg of quinidine) or vehicle (buffer or DMSO, respectively) was administered as a bolus to the perfusate reservoir 5 min prior to the addition of doxorubicin (464 μg). Perfusate and bile were collected during the perfusion, the liver was homogenized after the perfusion, and samples were analyzed by high-pressure liquid chromatography for doxorubicin and the major metabolite doxorubicinol. In the presence of GF120918, the biliary excretion of doxorubicin and doxorubicinol was decreased significantly without alterations in doxorubicin perfusate concentrations or concentrations of doxorubicinol liver concentrations. In the presence of quinidine, the biliary excretion of doxorubicinol was reduced significantly; however, doxorubicinol recovery in bile was not altered. The perfusate and liver concentrations of doxorubicinol were not altered by quinidine; doxorubicinol liver concentrations were increased. A series of pharmacokinetic models were evaluated incorporating perfusate, liver, and bile compartments to describe the disposition of doxorubicin and doxorubicinol in the isolated perfused rat liver. The model that best described these data, based on goodness-of-fit criteria, included first-order rate constants for all disposition processes. On the basis of this model, the rate-limiting process for doxorubicin and doxorubicinol elimination was biliary excretion. In the presence of GF120918, rate constants associated with doxorubicin and doxorubicinol canalicular egress were decreased, and other doxorubicinol disposition pathways were increased slightly. Quinidine was associated with a decrease in doxorubicin canalicular egress, doxorubicinol formation, and other doxorubicinol pathways. Pharmacokinetic modeling of the data supported the hypothesis that decreased biliary excretion of doxorubicin in the isolated perfused rat liver, as determined by mass-balance analysis, was due to interactions at the canalicular membrane. The present study further supports the utility of pharmacokinetic modeling in identifying sites of drug interactions within the hepatobiliary system. This approach may be particularly useful in predicting the effects of perturbations in hepatic translocation processes on the hepatobiliary disposition of drugs and derived metabolites.

INTRODUCTION

The treatment of cancer with chemotherapeutic agents is complicated by the phenomenon of MDR. MDR is due, in part, to the overexpression of a transmembrane glycoprotein, P-gp, which functions as an ATP-dependent transporter that pumps cytotoxic agents out of the cell, thus decreasing intracellular concentrations and limiting antitumor activity. One means of overcoming P-gp-mediated MDR is with the use of MDR modulators that inhibit the efflux of drugs from the cell by interacting with P-gp. Several MDR modulators have been identified in vitro, including verapamil (2), quinidine (3), cyclosporin A (4), cremaphor-EL (5), SDZ PSC-833 (5), and GF120918 (6). GF120918, an acridone carboxamide derivative, is an investigational drug currently in development by Glaxo Wellcome, Inc., as a MDR modulator. GF120918 has demonstrated remarkable potency for reversing MDR, with concentrations as low as 20 nm (6). Many chemotherapeutic agents are P-gp substrates and are eliminated primarily by hepatic processes. Doxorubicin, a P-gp substrate and an integral component of many cancer chemotherapy regimens, is eliminated primarily by hepatic metabolism and biliary excretion (7). Because doxorubicin is excreted primarily by the liver, altered hepatobiliary function could influence significantly the in vivo disposition of this agent. Patients with hepatic dysfunction receiving doxorubicin exhibit severe toxicity necessitating dosage reductions by as much as 50-75%, depending on the severity of liver disease (8).

The translocation of xenobiotics in the liver is influenced by several factors, including uptake into the hepatocyte, intracellular translocation, biotransformation, and egress into blood and/or bile. These processes may involve carrier-mediated mechanisms that are saturable and/or rate limiting. P-gp also is localized on the canalicular membrane of hepatocytes (9). P-gp functions as an ATP-dependent efflux pump in these cells, actively extruding substrates into bile. Interactions between chemotherapeutic agents and MDR modulators at the canalicular membrane may result in altered biliary excretion of these drugs in vivo. Cremophor EL, a surfactant used in the i.v. formulation of cyclosporin and paclitaxel, decreased both the hepatic clearance and biliary clearance of etoposide 4-5-fold in the isolated perfused rat liver (10). Biliary excretion of vincristine was decreased in the presence of verapamil in the isolated perfused rat liver (11). SDZ PSC-833, a nonimmunosuppressive cyclosporin D analogue, decreased the biliary clearance of both colchicine (3.8-fold) and doxorubicin (4.2-fold) in bile duct-cannulated rats in vivo (12). List et al. (13) reported decreased renal and nonrenal clearance of doxorubicin, resulting in a 55% increase in the area under the plasma concentration versus time curve in the presence of cyclosporin A.

The isolated perfused rat liver is an ideal model for examining alterations in the hepatobiliary disposition of substrates without the influence of metabolism/excretion by other organ systems (14). The disposition of doxorubicin in the isolated perfused rat liver has been examined by Skibba et al. (15); ~7% of the dose was excreted in bile over 3 h. The utility of this model system to examine the influence of MDR modulators on hepatobiliary disposition of chemotherapeutic...
agents also has been demonstrated. Watanabe et al. (11) reported that 50 μM verapamil selectively decreased the biliary excretion of vincristine; no differences in vincristine disposition were noted at 10 μM verapamil, whereas at 150 μM verapamil, the hepatic effects were not selective for vincristine (biliary excretion of taurocholate and bile flow also were reduced).

Often, the complexity of processes regulating the hepatobiliary disposition of drugs requires pharmacokinetic modeling techniques to elucidate specific sites of interaction that would not be evident based on mass-balance analysis. Interest in predicting drug/metabolite profiles and routes of hepatic excretion under normal conditions, as well as in the presence of interacting drugs or disease states that perturb translocation processes within the hepatobiliary system, has increased. Such predictions require an understanding of the mechanisms involved in the hepatic translocation of drugs/metabolites, as well as the rate-limiting steps governing these processes. The development of pharmacokinetic models based on in vitro data capable of describing these processes may improve the ability to predict alterations in hepatobiliary disposition of drugs/metabolites in response to such perturbations in vivo. The objective of the present research project was to elucidate the site(s) of interaction associated with doxorubicin and doxorubicinol hepatobiliary disposition in the presence of the MDR modulators GF120918 and quinidine in the isolated perfused rat liver using pharmacokinetic modeling techniques.

**MATERIALS AND METHODS**

**Chemicals.** Doxorubicin HCl, daunorubicin HCl, quinidine HCl monohydrate, and BSA were purchased from Sigma Chemical Co. (St. Louis, MO). GF120918 was generously supplied by Glaxo Wellcome Inc. (Research Triangle Park, NC). Doxorubicinol was graciously provided by Dr. Bruno Sardi (Pharmacia and Upjohn, Milan, Italy).

**Animals.** Male Sprague Dawley rats (Hilltop Laboratory Animals, Scottdale, PA) were used as liver donors (200–250 g) and as blood donors. Rats were housed individually in stainless-steel cages, were fed ad libitum, and were maintained on a 12-h light/dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill (Chapel Hill, NC).

**Isolated Perfused Liver Experiments.** Livers (9.54 ± 1.22 g) were isolated and perfused by standard techniques (16). Following anesthesia (urethane, 1.25 g/kg i.p.), the bile duct and portal vein were cannulated and the liver was perfused in situ with oxygenated Krebs-Ringer bicarbonate buffer maintained at 37°C. The liver was transferred to a 37°C perfusion chamber covered in aluminum foil, and perfusion was continued with 80 ml of recirculating oxygenated buffering containing 20% (v/v) heparin (100 U heparin sodium/mL) male rat blood at a flow rate of ~2 ml/min/g liver. The liver was allowed to equilibrate for ~10 min prior to addition of the dose. Liver viability was determined based on initial bile flow (>0.6 μL/min/g liver) and constant perfusion pressure (<15 cm H₂O). In representative livers, lactate dehydrogenase (17), alanineaminotransferase (18), and inflow and outflow O₂ concentration were measured.

Following equilibration of the liver, MDR modulator (0.3–3.0 mg of quinidine in 0.3 ml of DMSO or 16.8–480 μg of GF120918 in 0.5 ml of buffer containing 0.5% BSA) or vehicle (0.3 ml of DMSO or 0.5 ml of buffer containing 0.5% BSA, respectively) was added to the reservoir. Doxorubicin HCl (464 μg in buffer; 0.5 ml) was added to the reservoir 5 min after the addition of MDR modulator or vehicle. Previous experiments in isolated rat hepatocytes demonstrated that GF120918 and quinidine obtained equilibrium intracellular to extracellular concentrations within 5 min. Livers were perfused for 120 min after addition of doxorubicin; perfusate samples (~300 μL) were collected at timed intervals, and bile was collected in toto. The volume of bile was determined gravimetrically (specific gravity = 1.0), and plasma was harvested from perfusate samples. After perfusion, the liver was removed from the chamber, blotted, and weighed. Perfusate, bile, and liver were protected from light during collection and stored at ~20°C until analysis.

**Doxorubicin High-Pressure Liquid Chromatography Analysis.** Analysis of doxorubicin and doxorubicinol was performed according to previously published methods with slight modifications (19, 20). Bile samples (10 μL) were diluted with 10 μL of internal standard (daunorubicin, 25 μg/mL, 50 μL of H₂O, and 50 μL of 0.3 n H₃PO₄; mixed by vortex; and injected directly on column. Samples (150 μL) of perfusate or liver homogenate [1:2 (w/v), liver: H₂O] were diluted with 20 μL of internal standard (daunorubicin, 250 μg/mL) and 0.1 M boric acid (150 μL, pH 8.9) and mixed by vortex. Chloroform:2-propanol (4:1; 600 μL) was added to the sample, mixed by vortex for 1 min and centrifuged for 3 min. The organic layer was transferred to a clean polypropylene tube, and 100 μL of 0.1 n H₃PO₄ was added. The sample was mixed by vortex for 1 min and centrifuged for 3 min. The aqueous layer was removed for analysis by high-pressure liquid chromatography. Prepared perfusate, bile, and liver homogenate samples (20–50 μL) were eluted on a C18 column (250 mm × 4.6 mm, 5 μm; Altima C18; Alttech) at 1.0 ml/min with 20 mM KH₂PO₄·28% acetone/0.05% triethylamine (pH 3). Fluorescence of the column eluent was monitored continuously at excitation and emission wavelengths of 270 nm and 524 nm, respectively. Standard curves for doxorubicin (0.5–400 μg/mL, bile; 0.1–10 μg/mL, perfusate; and 30–1200 μg/g, liver homogenate) and doxorubicinol (0.1–30 μg/mL, bile; 0.05–8.0 μg/mL, perfusate; and 0.25–5 μg/g, liver homogenate) were prepared freshly on each day of analysis, were linear (r² ≥ 0.99), and had a percentage coefficient of variation of <10% for each standard concentration.

**Quinidine Analysis.** Concentrations of quinidine in perfusate were determined by the clinical toxicology laboratory at the University of North Carolina Hospitals using the Emit Quinidine Assay (Behring Diagnostics Inc., Cupertino, CA). The quantitation range of this assay was 0.5–8.0 μg/mL. The majority of the samples were diluted 10-fold prior to analysis, thus minimizing potential cross-reactivity with quinidine metabolites. Quinidine liver concentrations were predicted from measured quinidine perfusate concentrations and the hepatic distribution of quinidine in the isolated perfused liver at steady state (21).

**GF120918 Analysis.** Perfusion, bile, and liver homogenate samples were analyzed for GF120918 concentrations according to the established methods developed by Glaxo Wellcome, Inc.: Briefly, internal standard (GF126023X; 20 ng/mL; 100 μL) and a 200-μL aliquot of sample were mixed by vortex prior to the addition of ammonium hydroxide (1.05%; 600 μL). Samples were mixed by vortex, extracted with methyl-tert-butyl ether (2 mL), mixed by vortex for 15 min, and centrifuged for 10 min at room temperature. The organic layer was removed to a clean tube and evaporated to dryness with N₂ at 25°C. The residue was reconstituted with a 50 mM ammonium acetate:acetonicitrile (50:50) solution (150-μL). Prepared samples (50 μL) were eluted on a C18 column (250 × 4.6 mm, 5 μm; Keystone BDS Hypersil) at 1.0 ml/min with 50 mM ammonium acetate:acetonicitrile (55:45, v/v, pH 4.0). Postcolumn eluent was mixed with 0.25 N NaOH (1.0 ml/min) and monitored by fluorescence at excitation and emission wavelengths of 270 nm and 524 nm, respectively. Standard curves (5–1000 μg/mL) in bile, perfusate, and liver homogenate were prepared freshly, were linear (r² ≥ 0.99), and had a percentage coefficient of variation of <10% at each standard concentration.

**Data Analysis.** The percentage inhibition of doxorubicin(ol) biliary excretion, %I, was calculated as follows:

\[
%I = \left( \frac{\text{Doxorubicin(ol) amount in bile in the presence of modulator}}{\text{Doxorubicin(ol) amount in bile in the presence of vehicle}} \right) \times 100\%
\]

Inhibition constants, Eₘₐₓ and Eᵦᵦᵦᵦ, were estimated from mean percentage inhibition of doxorubicin(ol) biliary excretion versus mean GF120918 or quinidine liver concentration data by nonlinear least-squares regression (WinNonlin; Statistical Consultants, Inc., Apex, NC) according to the following equation:

\[
E = \frac{Eₘₐₓ \times C}{Eᵦᵦᵦᵦ + C}
\]

where C is the amount of GF120918 or quinidine recovered from the liver homogenate per g of liver at 120 min, Eᵦᵦᵦᵦ is the apparent concentration of GF120918 or quinidine in the liver homogenate at one-half maximal inhibition, and Eₘₐₓ is the estimated maximal percentage inhibition of doxorubicin(ol) biliary excretion.
The half-life of the doxorubicin distribution phase was calculated as follows:

\[ t_{1/2,\alpha} = \frac{\ln 2}{\alpha}, \]

where \( \alpha \) is the first-order disposition rate constant estimated by the method of residuals (22). The AUC was calculated with the trapezoidal method from 0 to 90 min.

Apparent hepatic clearance, \( CL_H \), and apparent biliary clearance, \( CL_B \), were calculated as follows:

\[ CL_H = \frac{V_{DOX}}{K_{S,U}} \times \frac{(K_{C,DOX} + K_H)}{(K_{C,DOX} + K_F + K_E)} \]

\[ CL_B = CL_H \times f_{ bile} \]

where \( f_{ bile} \) represents the fraction of the dose excreted in bile as doxorubicin.

Data from MDR modulator-treated groups were compared statistically to appropriate vehicle-treated groups using ANOVA and Dunnett's test (SAS, Inc., Cary, NC).

**Model Development.** A compartmental modeling approach was selected to describe the hepatobiliary disposition of doxorubicin and doxorubicinol in the isolated perfused rat liver. Both integrated and differential equations with combinations of Michaelis-Menten and first-order parameters were solved simultaneously with nonlinear least-squares regression (WinNonlin, Statistical Consultants, Inc., Apex, NC). Models included rate constants for doxorubicin sinusoidal uptake and egress, doxorubicinol formation, canalicular doxorubicin and doxorubicinol egress, and other doxorubicin and doxorubicinol pathways not supported directly by the data (presumably representing further biotransformation, protein binding, sequestration, and/or sinusoidal egress). Vehicle-treated livers for each MDR modulator group were used for model development. The goodness of fit for each model was assessed by visual examination of the distribution of residuals, rank and condition number of the matrix of partial derivatives (a rank less than the number of parameters indicates that there are insufficient data to estimate precisely all of the parameters; a condition number more than 10^6 indicates a high degree of colinearity between parameters in the model), and Akaike's Information Criterion (23).

A weighting scheme of \( 1/Y \) was used for doxorubicin and doxorubicinol biliary excretion rate versus time data. Perfusate concentration versus time data were weighted equally. The difference in weighting schemes for these data were used to account for the difference in the numerical magnitude of the data.

Data obtained from individual livers from all treatment groups (those treated with vehicle-buffer, GF120918, vehicle-DMSO, or quinidine) were analyzed by nonlinear least-squares regression according to the optimal model identified as described above. Statistical analyses were performed on parameter estimates from groups treated with vehicle-buffer and those treated with GF120918, and between groups treated with vehicle-DMSO and those treated with quinidine by ANOVA. The criterion for statistical significance was \( P < 0.05 \).

Sensitivity analysis for the optimal model was conducted by increasing or decreasing the initial parameter estimates 5-100-fold. Variability in parameter estimation after altering initial estimates was calculated from the average percentage difference compared to the original parameter estimate.

**RESULTS**

The initial bile flow in isolated perfused rat livers was greater than 0.6 \( \mu l/min/g \) liver, and the inflow perfusion pressure was less than 10 cm H\(_2\)O in all treatment groups. Perfusate lactate dehydrogenase and alanine aminotransferase levels measured in representative livers were \( \sim 20 \) units/g/h and \( \sim 9 \) units/g/h, respectively. Inflow oxygen delivery and oxygen consumption measured in a representative liver after 15 min of perfusion were within an acceptable range (1.063 ml/min and 0.271 ml/min, respectively) and did not decrease after 90 min of perfusion (1.080 ml/min and 0.267 ml/min, respectively). Gross observation of the livers in all treatment groups showed no signs of toxicity.

The majority (\( \sim 70\% \)) of GF120918 administered to the isolated
The excretion of doxorubicin was reduced 84% in the presence of 480 μg of MDR modulator GF120918 (GF) or 3.0 mg of quinidine (Q). The apparent terminal elimination rate (β) of doxorubicin was considered rapidly, with a t1/2,β of ~7.0 min in all treatment groups (Table 1). The concentration remained in perfusate after 30 min of perfusion and did not decrease further with perfusion time through 120 min.

Doxorubicin concentrations in perfusate declined in a biexponential manner (Fig. 1A). The initial doxorubicin distribution phase was rapid, with a t1/2,α of ~7.0 min in all treatment groups (Table 1). The apparent terminal elimination rate (β) of doxorubicin was considerably slower, and a terminal half-life could not be calculated reliably, because the perfusate collection period was much shorter than the apparent t1/2,β. Additionally, in some livers, doxorubicin perfusate concentrations after 90 min remained constant or increased slightly due to evaporation of plasma water from the isolated perfused liver system. Because of this artifact, doxorubicin perfusate concentrations obtained after 90 min were excluded from model evaluation. Statistical comparison of doxorubicin apparent hepatic clearance between livers receiving GF120918 and vehicle revealed no differences; no changes in the doxorubicin AUC were observed (Table 1). Likewise, no statistically significant differences in the apparent hepatic clearance between livers receiving quinidine and vehicle were noted (Table 1). In all of the treatment groups, doxorubicin perfusate concentrations were negligible.

In both vehicle groups, the cumulative amount of doxorubicin recovered in bile was ~37 μg (Fig. 1B). The cumulative biliary excretion of doxorubicin was reduced 84% in the presence of 480 μg of GF120918 and 72% in the presence of 3.0 mg of quinidine (Fig. 1B; Table 2). The total biliary output of doxorubicin also was reduced by 90% in the presence of 480 μg of GF120918 (Fig. 2A; Table 2); quinidine did not alter the cumulative amount of doxorubicin in bile (Fig. 2A; Table 2). Bile flow decreased slightly over the 120-min perfusion in both vehicle groups; neither MDR modulator altered bile flow.

The relationship between the percentage inhibition of doxorubicin or doxorubicinol biliary excretion versus the concentration of GF120918 remaining in the liver after the 120-min perfusion was nonlinear and described well by the simple Emax model defined above (Fig. 3A). The inhibition constants, Emax and EC50, for doxorubicin and doxorubicinol biliary excretion by GF120918, derived from the nonlinear least-squares regression analysis of the data this equation, were 90.5 ± 3.4% and 86.5 ± 9.8%, and 4.0 ± 0.6 μg/g and 2.4 ± 2.0 μg/g, respectively. Likewise, the relationship between the estimated concentration of quinidine remaining in the liver after the 120-min perfusion was nonlinear, with an estimated Emax of 100 ± 12.7% and an EC50 of 112 ± 39 μg/g (Fig. 3B).

The majority of the dose recovered was present in the liver homogenate as doxorubicin (Table 2). In all treatment groups, doxorubicin liver content accounted for 45–65% of the total dose. In the presence of GF120918 or quinidine, the liver content of doxorubicin at the end of the 120-min perfusion was not altered compared to the appropriate vehicle (Fig. 1C). The amount of doxorubicin remaining in the liver at the end of the perfusion accounted for ~1.5% of the dose (Table 2). GF120918 did not alter doxorubicin liver content (Fig. 2B; Table 2). At a 3.0-mg quinidine dose, the fraction of the dose remaining in the liver as doxorubicinol was increased significantly (Fig. 2; Table 2).

A series of pharmacokinetic models describing the hepatobiliary disposition of doxorubicin and doxorubicinol was evaluated. The model that best described the data comprised linear processes for doxorubicin sinusoidal uptake and egress, doxorubicinol formation, other doxorubicinol pathways, and doxorubicin and doxorubicinol canalicular egress (Fig. 4). Visual inspection of the model fit to the data showed that this model described the data well for all treatment groups (Fig. 5). Model-derived parameter estimates for the hepatobiliary disposition of doxorubicin and doxorubicinol in the absence and presence of GF120918 are listed in Table 3. As indicated by these data, the distribution of doxorubicin into the hepatocyte across the sinusoidal membrane was rapid. The rate-limiting process for doxorubicin or doxorubicinol elimination was biliary excretion represented by the canalicular rate constants Kc.DOX and Kc.DOXOL, respectively. No statistical differences in the rate constants governing doxorubicin sinusoidal uptake or egress, doxorubicinol formation, or the volume of doxorubicin distribution were detected in the presence of 480 μg of GF120918. The rate constants governing canalicular egress of both doxorubicin (Kc.DOX) and doxorubicinol (Kc.DOXOL) were decreased significantly (approximately 7-fold and 4-fold, respectively) in the presence of GF120918. In contrast, the rate constant describing other doxorubicinol disposition (Kc.DOXOL) was increased 2-fold from control in the presence of GF120918.

Parameter estimates describing doxorubicin and doxorubicinol hepatobiliary disposition in livers treated with vehicle-DMSO or 3 mg of quinidine are listed in Table 4. Similar to the vehicle-buffer and GF120918-treated livers, the rate constant associated with sinusoidal uptake of doxorubicin in the presence of vehicle-DMSO or quinidine was rapid; rate constants for doxorubicin and doxorubicinol canalicular egress were rate limiting. The rate constant associated with doxorubicin canalicular egress was decreased approximately 5-fold in quinidine-treated livers compared to livers treated with vehicle-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Doxorubicin pharmacokinetic parameters after administration to the isolated perfused rat liver in the presence and absence of MDR modulators, 480 μg of GF120918 (GF) or 3.0 mg of quinidine (Q)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>AUC(0−∞) (μmol × min/ml)</td>
</tr>
<tr>
<td>GF-vehicle</td>
<td>55 ± 12</td>
</tr>
<tr>
<td>GF</td>
<td>51 ± 15</td>
</tr>
<tr>
<td>Q-vehicle</td>
<td>55 ± 8</td>
</tr>
<tr>
<td>Q</td>
<td>48 ± 11</td>
</tr>
</tbody>
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* Data represent mean ± SD (n = 3–4), p < 0.05, treatment versus vehicle.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Percentage of the dose recovered as doxorubicin (DOX) or doxorubicinol (DOXOL) from isolated perfused rat livers in the presence or absence of MDR modulators, 480 μg of GF120918 (GF) or 3.0 mg of quinidine (Q)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix</td>
<td>Perfusion</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>GF-vehicle</td>
<td>18 (10)</td>
</tr>
<tr>
<td>GF</td>
<td>8.1 (2.3)</td>
</tr>
<tr>
<td>Q-vehicle</td>
<td>6.7 (4.1)</td>
</tr>
<tr>
<td>Q</td>
<td>7.3 (2.5)</td>
</tr>
</tbody>
</table>

* Data represent mean (SD); ND, not detectable.

p < 0.05, treatment versus vehicle.

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DISCUSSION

Previous studies indicated that GF120918 is a potent MDR-reversing agent that binds directly to P-gp and decreases the efflux of chemotherapeutic drugs out of resistant cells (6). The effect of GF120918 on the processes involved in hepatobiliary disposition of chemotherapeutic agents, and the influence of such alterations in hepatobiliary disposition on the systemic pharmacokinetics of the drugs, had not been determined. The purpose of the present investigation was to determine whether GF120918 altered the hepatobiliary disposition of a model chemotherapeutic agent, doxorubicin, and to what extent such alterations might affect doxorubicin pharmacokinetics. In addition, the effects of a first-generation MDR modulator, quinidine, on the hepatobiliary disposition of doxorubicin were examined and compared to those of GF120918.

After administration of doxorubicin to the isolated perfused rat liver in the presence of GF120918 or quinidine, the amount of doxorubicin excreted in bile was reduced markedly. Increasing amounts of GF120918 resulted in decreased doxorubicin biliary excretion. Likewise, GF120918 decreased the amount of doxorubicinol, the major doxorubicin metabolite, excreted in bile; quinidine did not decrease the amount of doxorubicinol excreted in bile. These results are consistent with the hypothesis that GF120918 and quinidine decrease the biliary excretion of doxorubicin by inhibiting the P-gp-mediated transport into bile. It is not clear whether doxorubicinol is a substrate for P-gp, although it is structurally similar. The potency with which GF120918 inhibited doxorubicin biliary excretion was similar to that of doxorubicin and suggests that the mechanism by which GF120918 inhibits doxorubicin biliary excretion is similar for doxorubicinol. It is possible that quinidine inhibited doxorubicin biliary excretion by a mechanism different, at least in part, from that of GF120918, because the biliary excretion of doxorubicinol was not altered by quinidine. However, alterations in the biliary excretion of doxorubicinol in the presence of quinidine may have been influenced by increased liver content of doxorubicinol. Both the doxorubicinol and doxorubicin bile:liver ratios in the presence of the high-dose quinidine were approximately 3.5-fold lower than in the vehicle group, suggesting that quinidine decreased the relative doxorubicinol biliary excretion to the same extent as doxorubicin.

In the presence of GF120918 or quinidine, the apparent hepatic clearance of doxorubicin was not altered. If decreased biliary excretion resulted in sequestration of doxorubicin in the liver, significant alterations in the amount of doxorubicin in the liver may not be detectable because the overwhelming majority of the recovered dose was in the liver homogenate.

The present study demonstrated that GF120918 and quinidine both altered the hepatobiliary disposition of doxorubicin. GF120918 was at least 40-fold more potent than quinidine, as demonstrated by a comparison of the estimated molar EC50 (7 versus 296 µM). Although doxorubicin is eliminated primarily by the liver, the almost complete inhibition of doxorubicin biliary excretion by GF120918 may not play a significant role in the overall elimination, because biliary excretion accounts for a minor portion of the total mass eliminated. In livers in which the P-gp transporter is overexpressed, decreased biliary excretion by MDR modulators may play a significant role in the systemic disposition of the chemotherapeutic agent (24). In the presence of quinidine, the amount of drug in the liver was not altered; however, an increase in the amount of doxorubicinol in the liver was noted. Doxorubicinol is an active cytotoxic agent, the mechanism of action of which is, at least in part, intracellular. These results suggest that doxorubicin doses may require adjustment in the presence of quinidine. However, it is unlikely that quinidine would be an effective in vivo MDR modulator, because the doses required to decrease biliary excretion would most likely result in severe cardiotoxicity.

A pharmacokinetic model was developed to describe the disposition of doxorubicin in the rat liver in the absence and presence of quinidine and GF120918. The hepatic disposition of doxorubicin and doxorubicinol was described best by a model comprising first-order processes for doxorubicin sinusoidal uptake and egress, doxorubicinol formation, other doxorubicin pathways, and doxorubicin and doxorubicinol canalicular egress. Selection of this model was based on complete rank (i.e., all parameters supported by the data), uncorrelated parameter estimates (as indicated by condition number <10⁶), lowest Akaike's Information Criterion compared to models with full rank and acceptable condition numbers, and minimal systematic bias.
doxorubicin distribution in perfusate. Canalicular egress of DOXOL: K_{C,DOX}. Volume of order rate constant for other DOXOL disposition constant for DOXOL formation; K_{0,DOXOL} > first-order rate constant for sinusoidal uptake of DOX: K_{SU,DOX}. First-order rate constant for sinusoidal egress (DOXOL) in the isolated perfused rat liver. Subscripts denote perfusate (P), liver (L), and bile (B).

Parameter designations are as follows: K_{S,U}, first-order rate constant for sinusoidal uptake of DOX; K_{S,E}, first-order rate constant for sinusoidal egress of DOX; K_{C,DOX}, first-order rate constant for canalicular egress of DOX; K_{F}, first-order rate constant for DOXOL formation; K_{C,DOXOL}, first-order rate constant for other DOXOL disposition pathways; K_{D,DOXOL}, first-order rate constant for canalicular egress of DOXOL; V_{DOX}, volume of doxorubicin distribution in perfusate.

Fig. 4. Model scheme describing the disposition of doxorubicin (DOX) and doxorubicinol (DOXOL) in the isolated perfused rat liver. Subscripts denote perfusate (P), liver (L), and bile (B). Parameter designations are as follows: K_{S,U}, first-order rate constant for sinusoidal uptake of DOX; K_{S,E}, first-order rate constant for sinusoidal egress of DOX; K_{C,DOX}, first-order rate constant for canalicular egress of DOX; K_{F}, first-order rate constant for DOXOL formation; K_{C,DOXOL}, first-order rate constant for other DOXOL disposition pathways; K_{D,DOXOL}, first-order rate constant for canalicular egress of DOXOL; V_{DOX}, volume of doxorubicin distribution in perfusate.

Fig. 3. Percentage of inhibition of doxorubicin and doxorubicinol (A, inset) biliary excretion versus GF120918 (A) or quinidine (B) liver concentration at 120 min after administration of 464 μg of doxorubicin. Data are means (bars, SD) where appropriate (n = 1-4). Lines represent nonlinear least-squares regression analysis.

in residual error. This model was based on the following assumptions: (a) doxorubicin transport across the sinusoidal membrane was bidirectional; (b) doxorubicinol formation was irreversible, and only doxorubicin in the liver compartment was metabolized; (c) additional pathways for doxorubicinol disposition existed (e.g., metabolism and binding) with net unidirectional loss; and (d) rate constants for doxorubicin and doxorubicinol were unidirectional and associated with the amount of compound in the liver compartment. These assumptions were based on the physiology of the liver and characteristics of doxorubicin and doxorubicinol. Although doxorubicinol is a cation that is ionized at physiological pH, it is taken into cells primarily by passive diffusion (25-27). Doxorubicinol, the major metabolite of doxorubicin in the rat, is formed via cytoplasmic aldo-keto reductases and can undergo further biotransformation to form aglycone metabolites and/or glucuronide and sulfate conjugates (28). In normal liver cells, doxorubicin is localized in the nucleus (29, 30); because doxorubicinol differs from doxorubicin only by the reduction of a keto group, nuclear sequestration is likely to occur. Drug transport into bile usually occurs against a large concentration gradient, and it is presumed that compounds gain access to bile primarily by active transport processes (31).

On the basis of the parameter estimates derived from the model, initial distribution of doxorubicin into the liver was rapid relative to the other parameters; elimination was rate limited by biliary excretion. The rate constant associated with other doxorubicinol disposition was rapid also. The rate-limiting step was canalicular egress of doxorubicinol.

In the presence of GF120918, the rate constants associated with doxorubicin and doxorubicinol canalicular egress were reduced significantly. Pharmacokinetic modeling of data obtained from quinidine-treated livers also resulted in a significant decrease in the rate constant for doxorubicin canalicular egress; the canalicular egress rate constant for doxorubicinol was not altered. These results support the hypothesis that decreased recovery of doxorubicin in bile in the isolated perfused rat liver, as determined by conventional mass-balance analysis, was due to an interaction at the canalicular membrane. In canicular plasma membrane vesicles, uptake of daunorubicin was decreased by 4.5-fold and 4.2-fold in the presence of doxorubicin and quinidine, respectively; these results were attributed to a competitive interaction for P-gp-mediated uptake (9). Daunorubicin efflux by CH9/C5 cells was inhibited in the presence of GF120918 at concentrations as low as 20 nM; P-gp photoaffinity labeling by [3H]azidopine was inhibited by GF120918 (6).

Analysis of data from GF120918-treated livers with the model also showed a 2-fold increase in the rate constant associated with other doxorubicinol pathways. It is presumed that this rate constant describes subsequent doxorubicinol biotransformation, protein binding, sequestration, and/or sinusoidal egress. This rate constant is defined by multiple processes that are not supported directly by data; alterations in this rate constant are due to changes in mass balance within the system. In addition, sensitivity analysis of this parameter suggests that this rate constant cannot be precisely determined with the present data set. Therefore, a clear, mechanistic explanation for the slight increase in this rate constant is not possible. GF120918 may have increased the sinusoidal egress of doxorubicinol, but this cannot be confirmed, because doxorubicinol perfusate concentrations were below assay sensitivity. In beagle dogs pretreated with GF120918 (25...
Fig. 5. Doxorubicin perfusate concentration (μg/ml) versus time (•), doxorubicin biliary excretion rate (μg/min) versus time (●), and doxorubicinol biliary excretion rate (μg/min) versus time (▲) from a representative liver in the G120918-vehicle-treated group (A), G120918-treated group (B), quinidine-vehicle-treated group (C), and quinidine-treated group (D). Solid lines represent the computer-generated best fit of the compartmental model equations to the data.

Table 3 Model parameter estimates for the disposition of doxorubicin (DOX) and doxorubicinol (DOXOL) in livers from vehicle-buffer- and 480 μg of GF120918-treated groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle-Buffer</th>
<th>G120918</th>
</tr>
</thead>
<tbody>
<tr>
<td>KE,DOX</td>
<td>7.22 ± 2.34</td>
<td>8.94 ± 1.76</td>
</tr>
<tr>
<td>KE,DOXOL</td>
<td>1.51 ± 0.63</td>
<td>0.55 ± 0.107</td>
</tr>
<tr>
<td>KE,DOX</td>
<td>0.219 ± 0.088</td>
<td>0.030 ± 0.027</td>
</tr>
<tr>
<td>KE,DOXOL</td>
<td>1.90 ± 0.88</td>
<td>1.35 ± 1.22</td>
</tr>
<tr>
<td>KE,DOX</td>
<td>4.25 ± 0.56</td>
<td>8.33 ± 1.62</td>
</tr>
<tr>
<td>KE,DOXOL</td>
<td>0.0710 ± 0.0340</td>
<td>0.0190 ± 0.0040</td>
</tr>
<tr>
<td>VC,DOX</td>
<td>192 ± 69</td>
<td>154 ± 75</td>
</tr>
</tbody>
</table>

Table 4 Model parameter estimates for the disposition of doxorubicin (DOX) and doxorubicinol (DOXOL) in livers from vehicle-DMSO- and 3.0 mg quinidine-treated groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle-DMSO</th>
<th>Quinidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>KE,DOX</td>
<td>8.72 ± 2.00</td>
<td>10.5 ± 1.8</td>
</tr>
<tr>
<td>KE,DOXOL</td>
<td>0.626 ± 0.098</td>
<td>0.416 ± 0.257</td>
</tr>
<tr>
<td>KE,DOX</td>
<td>0.130 ± 0.033</td>
<td>0.026 ± 0.008</td>
</tr>
<tr>
<td>KE,DOXOL</td>
<td>0.941 ± 0.323</td>
<td>0.417 ± 0.322</td>
</tr>
<tr>
<td>KE,DOX</td>
<td>3.92 ± 0.13</td>
<td>2.27 ± 1.06</td>
</tr>
<tr>
<td>KE,DOXOL</td>
<td>0.070 ± 0.043</td>
<td>0.091 ± 0.105</td>
</tr>
<tr>
<td>VC,DOX</td>
<td>136 ± 39</td>
<td>145 ± 16</td>
</tr>
</tbody>
</table>

mg/kg/day for 4 day), the doxorubicinol area under the plasma versus time curve was increased ~2-fold after a single i.v. bolus dose of doxorubicin (1.5 mg/kg; Ref. 32).

In contrast to G120918-treated livers, quinidine-treated livers showed a 2-fold decrease in the rate constant associated with other doxorubicinol pathways. One explanation for this observation may be that quinidine inhibits metabolism of doxorubicinol. Both quinidine and doxorubicinol are metabolized by microsomal enzymatic systems (28, 33).

The estimated doxorubicin volume of distribution from the pharmacokinetic model was ~2-fold greater than the volume of perfusate in these experiments. This may be explained, in part, by 25% partitioning into RBCs (34); however, the perfusion medium used in the present study was composed of only 20% blood. Doxorubicin may distribute into a rapid equilibrium liver compartment that is incorporated into the perfusate compartment.

Rate constants associated with doxorubicin sinusoidal uptake and egress were not altered in the presence of G120918 or quinidine. This is consistent with previous observations in isolated hepatocytes; [3H]daunorubicin uptake and egress across the sinusoidal membrane in the presence of G120918 or verapamil were not altered (35). The current pharmacokinetic model indicates that doxorubicinol formation was not altered significantly by G120918 or quinidine. Likewise,
doxorubicinol formation was not altered by GF120918 or quinidine in the 9000 × g supernatant fraction of rat liver homogenate (36). Interestingly, the average doxorubicinol formation rate constant in the isolated perfused rat liver, as determined by mass-balance analysis, was due to interactions at the canalicular membrane. This approach may be particularly useful in predicting the effects of perturbations in hepatic translocation processes on the hepatobiliary disposition of drugs and derived metabolites. The clearance of a compound from the systemic circulation comprises numerous processes. The relative contribution of each individual process to total clearance will dictate whether alterations in a given process will result in a change in the overall disposition. In the present study, it was demonstrated clearly that MDR modulators alter the overall hepatobiliary disposition of a model chemotherapeutic agent.

REFERENCES

Effect of Multidrug Resistance Modulators on the Hepatobiliary Disposition of Doxorubicin in the Isolated Perfused Rat Liver

Catherine L. Booth, Kenneth R. Brouwer and Kim L. R. Brouwer