Increased Oral Bioavailability of Paclitaxel by GF120918 in Mice through Selective Modulation of P-glycoprotein

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ABSTRACT

Previous studies in mice with disrupted mdr1a P-glycoprotein genes have shown that the oral bioavailability of paclitaxel is very low because of the presence of this drug-transporting protein in the intestinal wall. Additional studies with cyclosporin A have shown that this P-glycoprotein-inhibiting agent is able to increase the bioavailability of paclitaxel in mouse models and in patients. However, the potential immune-suppressive side effects of cyclosporin A renders this compound less suitable for chronic use in cancer patients. In this paper we present the results obtained with GF120918, an experimental P-glycoprotein inhibitor, on the oral bioavailability of paclitaxel in both wild-type and mdr1ab knockout mice. GF120918 (25 mg/kg) was administered p.o. by gavage 15 min or 2 h before oral or i.v. dosing with GF120918, an experimental P-glycoprotein inhibitor, respectively. Paclitaxel plasma levels were quantified by high-performance liquid chromatography. GF120918 increased the plasma values for areas under the concentration-time curve; plasma AUC2 after oral dosing was 6-fold higher in these mice than in wild-type mice (from 11 to 35%). In the absence of P-glycoprotein, first-pass metabolism [e.g., by cytochrome P450 isoenzymes to 3-p-hydroxy-paclitaxel and 6α-hydroxy-paclitaxel (11–13)] becomes an important factor limiting the oral bioavailability of paclitaxel. This is illustrated by the observation that the fraction of unchanged paclitaxel recovered from the feces was reduced from 86% of the dose in wild-type mice to <2% in knockout mice (10). Thus, despite the virtually complete absorption from the gastro-intestinal tract, the bioavailability in the knockout mice does not approach 100%. The plasma levels of the hydroxylated metabolites remained below the limit of detection, whereas their contribution in the fecal excretion was high [25–30% of the administered paclitaxel dose (10)]. Apparently, these

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2 The abbreviations used are: AUC, area under the concentration-time curve; Cl, clearance; Cmax, maximum plasma level.
metabolites are efficiently excreted into the bile after their formation and do not reach the systemic circulation.

A subsequent study with PSC833, a cyclosporin D analogue and potent P-glycoprotein-blocker, demonstrated the potential of using P-glycoprotein-blockers to increase paclitaxel plasma levels after oral administration (14). The plasma AUC using the PSC833-paclitaxel combination was even higher than that achieved in mdrla knockout mice receiving paclitaxel as a single agent, suggesting that the gain in systemic drug levels was also attributable to the inhibition of other drug-elimination pathways (metabolic enzymes or other transporters). Although not as potent as PSC833, cyclosporin A is a registered drug and is thus more readily available for clinical studies. After showing the usefulness of cyclosporin A in a preclinical setting (15) a subsequent clinical study demonstrated the potential usefulness of this approach in humans (16, 17). However, the use of cyclosporin A for long-term oral dosing may be hindered by the immune suppressive effect of this agent. Consequently, we are currently exploring other agents that block P-glycoprotein for increasing the oral bioavailability of paclitaxel.

Several other P-glycoprotein-blockers are under development [e.g., GF120918 (18, 19), X9576 (22), and VX710 (23, 24)], however, primarily with the purpose to improve the treatment of P-glycoprotein-expressing multidrug resistant tumors. GF120918 is an acridonecarboxamide derivative and has been shown to be a potent blocker of P-glycoprotein in tumor cells in vitro and in vivo (18). This paper describes a study with GF120918 to enhance the oral bioavailability of paclitaxel in wild-type mice. The current studies have also included the use of mice carrying a disruption of both murine isoforms (mdrla and mdrlb) of drug transporting P-glycoproteins (mdrlab knockout mice; Ref. 25), which have recently become available in a 99% pure FVB background. By integration of groups of both wild-type and mdrlab knockout mice receiving oral or i.v. paclitaxel, with or without GF120918, insight is obtained into both the potency as well as the selectivity for P-glycoprotein-inhibition of this agent.

MATERIALS AND METHODS

Animals. Female wild-type FVB mice and 99% pure FVB mdrlab knockout mice, 9–14 weeks of age, were used throughout the experiments. Mice were allowed to take water and food ad libitum.

Their body weights ranged from 18.7 to 28.4 g, and were evenly distributed throughout the different test groups. Animals were maintained and handled in accordance with institutional guidelines based on Dutch law.

Drugs. Taxol® (paclitaxel 6 mg/ml, formulated in Cre- mophor EL;ethanol 1:1; v/v) was obtained from Bristol Myers Squibb (Princeton, NJ). Paclitaxel, pure compound, originated from Sankyo (Tokyo, Japan). GF120918A (HCl salt) was obtained from Glaxo Wellcome (Research Triangle Park, NC).

Dosing Solutions. Paclitaxel solutions for oral administration were prepared by a 6-fold dilution of the pharmaceu- tical formulation with saline (Braun Emmer Compascuum, The Netherlands). The final concentration was 1 mg/ml. To circumvent the Cremophor EL-caused nonlinear pharmacokinetic behavior of paclitaxel (6), the stock solution of paclitaxel for i.v. dosing was prepared by dissolving 30 mg of pure compound in 2.5 ml of ethanol (Merck, Darmstadt, Germany) and 2.5 ml of polysorbate 80 (Sigma, St. Louis, MO). This stock solution containing 6 mg/ml of paclitaxel was stable for at least 1 year if stored at 4°C. Before injection, this stock solution was diluted 6-fold with saline to a final concentration of 1 mg/ml and used within 4 h.

GF120918 suspensions were freshly prepared the day before each experiment. A portion of 40 mg of GF120918A was accurately weighed and transferred into a glass tube. The powder was suspended in 7.5 ml of water for injection (NPB) and transferred into a polypropylene Falcon tube (Becton Dickinson Labware, Franklin Lakes, NJ) containing 7.5 ml of concentrated stock vehicle (10 g/liter of hydroxypropyl methyl cellulose (100 centipoises; Sigma) and 2% Tween 80 (Sigma) in water (NPB). The suspension was mixed for 10 min, and then further dispersed using a Polytron PT1200 homogenizer for 2 min. The suspension was kept protected from light and was stirred continuously. The final concentration was 2.75 mg/ml of GF120918A (salt) corresponding to 2.5 mg/ml of GF120918 (active substance).

The vehicle solution of GF120918 consisted of the concentrated stock vehicle diluted 1:1 (v/v) with water.

Administration Routes. Oral drug or vehicle administrations were given by gavage. i.v. drug administrations were done by injection with a 29-gauge needle into a lateral tail vein using 300-μl syringes. Animals were placed under a heating lamp prior to i.v. drug administration. No anesthetics were used during these procedures.

Test Groups. Eight different test groups, four groups of wild-type mice (A–D) and four groups of mdrlab knockout mice (E–H) were included in this study. Groups A and E received GF120918 vehicle p.o. and oral paclitaxel within 10–20 min. Groups B and F received GF120918 p.o. and oral paclitaxel within 10–20 min. Groups C and G received GF120918 vehicle p.o. and i.v. paclitaxel after 2 h (±15 min). Groups D and H received GF120918 p.o. and i.v. paclitaxel after 2 h (±15 min).

The dose level of paclitaxel was 10 mg/kg in all groups. The dose level of GF120918 was 25 mg/kg. Groups receiving oral paclitaxel consisted of at least six animals/time point, whereas at least three animals/time point were used when paclitaxel was administered i.v.

Blood sampling was performed at the following approximate time points after the administration of paclitaxel (in parentheses, for knockout mice only): groups A and E, 0.5, 1, 2, 3, 4, 6, 8, 12, and 16, (24) h; groups B and F, 0.5, 1, 2, 3, 4, 6, 8, 12, and 16, (24) h; groups C and G, 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 12, (16 and 24) h; and groups D and H, 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, and 24 h.

Sample Collection and Handling. Blood was obtained by cardiac puncture under anesthesia with methoxyflurane. Potassium EDTA was used as an anticoagulant and the plasma obtained after centrifugation (5 min, 3000 x g) was stored at −20°C until analysis.

Analytical Methods. Mouse plasma samples were diluted with blank human plasma. Paclitaxel was determined using a validated high-performance liquid chromatography-UV
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The plasma levels of GF120918 have been analyzed using a newly developed assay. This assay is based on high-performance liquid chromatography with fluorescence detection after liquid-liquid extraction with tert-Butyl methyl ether. The lower limit of quantitation is 22.4 ng/ml using 50 μl of mouse plasma.

Pharmacokinetic Calculations. Pharmacokinetic parameters were calculated by noncompartmental methods using the software package Quattro Pro (Corel Corp., 1996; version 6.02). The plasma AUC from time 0 to the last sampling point was calculated by the linear trapezoidal rule with the formula:

\[ AUC = \sum_{i=2}^{n} \frac{\text{Concentration}_i \cdot (\text{Δtime}_{i-1} + \text{Δtime}_i)}{2} \]

with Δtime\(_n\) = 0.

The SE of the AUC was calculated with the law of propagation of errors using the formula:

\[ \text{SE}_{AUC} = \left( \sum_{i=2}^{n} \text{SE}_i \right) \left( \frac{(\text{Δtime}_{i-1} + \text{Δtime}_i)^2}{4} \right) \]

The elimination constant (k) and the SE\(_k\) of the elimination constant were calculated by linear regression analysis of the log (concentration) versus time data points of the final log-linear part of the concentration-time curve. The terminal half-life (t\(_{1/2}\)) and the SE\(_{1/2}\) were calculated by the formulas t\(_{1/2}\) = ln(2)/k and SE\(_{1/2}\) = t\(_{1/2}\)SE\(_k\)/k. The plasma clearance (Cl) after i.v. paclitaxel administration and the SE were calculated using the formulas Cl = Dose/AUC\(_{i.v.}\) and SE\(_{Cl}\) = ClSE\(_{AUC}\)/AUC.

RESULTS

Consistent with previous results the plasma levels of paclitaxel in wild-type mice receiving the drug by the oral route remained very low (Fig. 1). The plasma levels hardly exceeded the 0.1 μM (~85 ng/ml) level, which is considered of therapeutic relevance (27). The plasma AUC of paclitaxel, however, increased significantly by 6.6-fold (P < 0.001; Table 1) when GF120918 was given p.o. 10–20 min before the oral administration of paclitaxel. The C\(_{max}\) increased to 770 ± 120 ng/ml, and the plasma concentration remained above 85 ng/ml level for >6 h. The administration of GF120918 also increased the plasma AUC of paclitaxel when this drug was given i.v. to wild-type mice, but only by a factor 1.4 (P < 0.01). This increased AUC was attributable to a less-rapid decline of the plasma concentration. The C\(_{max}\) of i.v.-administered paclitaxel was similar with or without GF120918, suggesting that this P-glycoprotein-blocker does not affect the central distribution volume of paclitaxel. However, the plasma concentration time curves started to diverge after 1 h and were ~2-fold higher 2 h after the administration of paclitaxel. The terminal half-life was ~2-fold longer in the mdr1ab knockout mice relative to wild-type controls. Taking into account this effect of GF120918 on the CI of paclitaxel, GF120918 very significantly increased the oral bioavailability of paclitaxel in wild-type mice from 8.5% to 40.2% (P < 0.001).

The plasma pharmacokinetic behavior of paclitaxel in the mdr1ab knockout mice, when given by the oral route, was not altered by GF120918 because the plasma concentration time curves in these groups were overlapping. The plasma pharmacokinetics of paclitaxel in knockout mice after i.v. administration was also similar with or without GF120918. After oral

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administration of paclitaxel, the results in wild-type mice receiving GF120918 showed a trend toward a higher AUC compared with knockout mice. However, the difference is small and did not reach statistical significance \( (P = 0.05) \).

The maximum plasma level of GF120918 ranged between 150 and 250 ng/ml and was reached at 2 h after oral administration. These levels were consistent with previous reports in mice \( (18) \).

### DISCUSSION

This study shows that GF120918 is able to increase the oral bioavailability of paclitaxel in mice significantly. The plasma AUC of paclitaxel achieved in wild-type mice when given with GF120918 became comparable with that in knockout mice. Moreover, mdr1b knockout mice, which lack both murine isoforms of drug transporting P-glycoproteins, provide an elegant in vivo test system to monitor the selectivity of P-glycoprotein inhibitors on the pharmacokinetics of substrate drugs. Apparently, next to the inhibition of P-glycoprotein, GF120918 became comparable with that in knockout mice. We have shown previously that a disruption of the mdr1a and mdr1b P-glycoprotein genes in mice does not affect hepatobiliary excretion of paclitaxel \( (25) \). Apparently, other transporters in the biliary canalicular membrane compensate for the absence of drug transporting P-glycoproteins. This result is different from doxorubicin, which was much less excreted in the bile of mdr1a knockout mice \( (31) \). In line with this result, a functional blockade of P-glycoprotein by GF120918 also resulted in a significant reduction of doxorubicin in an isolated perfused rat liver model \( (32) \).

The difference in the plasma pharmacokinetics of paclitaxel between wild-type and mdr1b knockout mice is mainly caused by the activity of P-glycoprotein in the gut wall, which is of mdr1a type. The direct excretion of paclitaxel from the blood, via the intestinal epithelium into the gut lumen, is markedly decreased in mdr1a \( (and\ mdr1b) \) knockout mice relative to wild-type controls \( (10, 25) \). Conversely, (re)uptake of paclitaxel from the gut lumen (after biliary excretion or oral administration) into the systemic circulation is very effective in knockout mice, because the fecal excretion of unchanged drug amounts to only 2% of the dose \( (10) \). The finding that the plasma concentration of paclitaxel in wild-type mice receiving GF120918 remains similar to that found in mdr1b knockout mice for at least 12 h after i.v. administration of paclitaxel suggests that GF120918 blocks P-glycoprotein in the intestines during this entire period.

GF120918 is a very poorly water soluble compound for which no suitable i.v. formulation has become available. The oral formulation used throughout this study is a finely dispersed suspension. Clinical studies with GF120918 aimed to investigate its potential as a reversal agent in the treatment of multidrug-resistant tumors have demonstrated substantial interpatient variability in the plasma levels of GF120918 \( (33) \). The variable plasma levels of GF120918 after oral administration, which may be caused by its poor aqueous solubility, have led to a postponement in this part of the drug development program. However, for the purpose of using this compound to increase the oral bioavailability of paclitaxel, the variation in the plasma levels of GF120918 may not be a major obstacle. The local concentration of GF120918 in the intestinal lumen is probably much more important and was apparently high enough to completely block intestinal P-glycoprotein in mice. Because the rate of disappear-

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**Table 1** Summary of pharmacokinetic parameters (noncompartmental analysis)

<table>
<thead>
<tr>
<th>Group</th>
<th>GF120918 (Oral)</th>
<th>Paclitaxel dose/route (mg·kg⁻¹)</th>
<th>AUC (ng·ml⁻¹·h)</th>
<th>( F ) (%)</th>
<th>( C_{\text{max}} ) (ng·ml⁻¹)</th>
<th>CI (liter·h⁻¹·kg⁻¹)</th>
<th>( t_{1/2} ) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Vehicle</td>
<td>10/p.o.</td>
<td>408 ± 36</td>
<td>8.5 ± 0.8</td>
<td>118 ± 20</td>
<td>1.95 ± 0.64</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Drug</td>
<td>10/p.o.</td>
<td>2650 ± 145*</td>
<td>40.2 ± 1.9*</td>
<td>770 ± 120</td>
<td>1.98 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Vehicle</td>
<td>10/i.v.</td>
<td>4783 ± 424</td>
<td>2.09 ± 0.19</td>
<td>1.18 ± 0.05</td>
<td>2.19 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Drug</td>
<td>10/i.v.</td>
<td>6600 ± 306*</td>
<td>1.52 ± 0.07</td>
<td>2.19 ± 0.19</td>
<td>2.19 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>Knockout mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Vehicle</td>
<td>10/p.o.</td>
<td>2057 ± 158</td>
<td>36.1 ± 2.6</td>
<td>553 ± 130</td>
<td>2.59 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Drug</td>
<td>10/p.o.</td>
<td>2252 ± 112*</td>
<td>35.4 ± 2.4</td>
<td>530 ± 110</td>
<td>2.69 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Vehicle</td>
<td>10/i.v.</td>
<td>5701 ± 402</td>
<td>1.73 ± 0.12</td>
<td>2.00 ± 0.11</td>
<td>2.19 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Drug</td>
<td>10/i.v.</td>
<td>6361 ± 437</td>
<td>1.57 ± 0.11</td>
<td>1.95 ± 0.11</td>
<td>2.19 ± 0.19</td>
<td></td>
</tr>
</tbody>
</table>

* \( P < 0.001 \) (A versus B).

\( P < 0.01 \) (C versus D).

\( P = 0.05 \) (E versus F) as determined by Student \( t \) test.
ance of the P-glycoprotein inhibitor from the gut may be relevant for the duration of blocking P-glycoprotein, it may be speculated that a gradual dissolution of GF120918 in the intestinal tract, such as a slow release formulation, could even be advantageous to increase the oral bioavailability of paclitaxel.

Intrinsic or acquired (multi)drug resistance remains an important problem in the chemotherapeutic treatment of cancer patients. P-glycoprotein-inhibitors like GF120918 have been developed by drug discovery programs aimed at finding agents that block P-glycoprotein in tumor tissues and, by that, reversing P-glycoprotein-mediated multidrug resistance. It is generally anticipated that, within the coming years, studies using these compounds in combination with anticancer drugs with a high affinity for P-glycoprotein will resolve the issue of whether P-glycoprotein is of clinical relevance in multidrug-resistant (solid) tumors and whether P-glycoprotein blockers contribute to an improvement in treatment response. However, our recently obtained insight into the pharmacological role of P-glycoprotein in normal tissues, which fulfills a barrier function [e.g., the gut lumen-blood barrier (6, 34), the blood-brain barrier (35, 36), and the blood-fetus barrier (37, 38)], makes this class of compounds potentially very useful when increased levels of P-glycoprotein substrate drugs at these sanctuary sites are required for therapeutic reasons. On the basis of the promising results with GF120918 in this study, a study in patients receiving the combination of oral GF120918 and oral paclitaxel has been initiated. These studies will determine whether the combination provides sufficiently high and reproducible plasma levels of paclitaxel to allow oral administration of this drug.

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