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 mdrla 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 render 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 mdrlab knockout mice. GF120918 (25 mg/kg) was administered p.o. by gavage 15 min or 2 h before oral or i.v. dosing of paclitaxel, respectively. Paclitaxel plasma levels were quantified by high-performance liquid chromatography. GF120918 increased the plasma values for areas under the concentration-time curve of oral paclitaxel in wild-type mice by 6.6-fold from 408 to 2701 ng/ml h. Calculated relative to their respective values for area under the concentration-time curve after i.v. administration, GF120918 increased the oral bioavailability of paclitaxel in wild-type mice from 8.5 to 40.2%. The plasma pharmacokinetics of paclitaxel in mdrlab knockout mice was not altered by GF120918, whereas the pharmacokinetics of paclitaxel in wild-type mice receiving GF120918 became comparable with mdrlab knockout mice. This result indicates that GF120918 at this dose-level selectively and completely blocks P-glycoprotein in the intestines and does not notably interfere in the elimination of paclitaxel by metabolism or other transporters. On the basis of this result, GF120918 has been selected for additional study in humans.

INTRODUCTION

Paclitaxel (Taxol®, Paxene) has become an established drug in the treatment of various human malignancies (see reviews by Huizing et al., Ref. 1, and Rowinsky and Donehower, Ref. 2). In current clinical practice, the drug is administered by i.v. infusion. Because of its very limited aqueous solubility, the drug is formulated at 6 mg/ml in a mixture of Cremophor EL and ethanol (1:1; v/v). Thus, patients who receive paclitaxel concomitantly receive substantial amounts of Cremophor EL, resulting in plasma levels of Cremophor EL of about 1% of the plasma volume (3). The excipient Cremophor EL is believed to play a role in the (severe) hypersensitivity reactions occasionally observed in patients while on paclitaxel therapy (4, 5). Moreover, Cremophor EL also causes the nonlinear pharmacokinetic behavior of paclitaxel (6–8).

In addition to avoiding the systemic effects of Cremophor EL, the availability of an oral drug formulation would offer several additional advantages over i.v. dosing, including patients’ preferences for oral administration, elimination of the need for frequent visits to the outpatient clinic, and easier chronic administration. Initially, the oral route was thought to be impractical because of very low bioavailability in preclinical studies (9). Additional studies in mdrla P-glycoprotein knockout mice revealed that this drug efflux pump is the major limiting factor in drug uptake from the intestines because the plasma AUC2 after oral dosing was 6-fold higher in these mice than in wild-type controls (10). Because the clearance of paclitaxel in knockout mice was almost 2-fold lower, the oral bioavailability (AUCoral/AUCi.v.×100%) increased by 3.5-fold (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
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 mdr1a 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), LY335979 (20, 21), XR9576 (22), and VX710 (23, 24)], however, primarily with the purpose to improve the treatment of P-glycoprotein-expressing multidrug resistant tumors. GF120918 is an acridinecarboxamide-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 (mdr1a and mdr1b) of drug transporting P-glycoproteins (mdr1ab knockout mice; Ref. 25), which have recently become available in a 99% pure FVB background. By integration of groups of both wild-type and mdr1ab 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 mdr1ab 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 Cremophor 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). 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, 3, 4, 6, 8, 10, 12, and 16, (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 × 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
was calculated by the linear trapezoidal rule with the formula:

The plasma AUC from time 0 to the last sampling point was 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:

with Δtime_{i} = 0.

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

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_{t} were calculated by the formulas \( t_{1/2} = \ln(2)/k \) and \( \text{SE}_{t} = t_{1/2} \text{SE}_{k}/k \). The plasma clearance (Cl) after i.v. paclitaxel administration and the SE were calculated using the formulas \( \text{Cl} = \text{Dose} / \text{AUC}_{i.v.} \) and \( \text{SE}_{\text{Cl}} = \text{Cl} \text{SE}_{\text{AUC}} / \text{AUC} \).

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

The oral bioavailability (F) and the SE_{F} were calculated using the formula \( F = AUC_{i.v.} / AUC_{i.v.} \) and \( \text{SE}_{F} = F \cdot (\text{SE}_{AUC, i.v.} / AUC_{i.v.})^2 + (\text{SE}_{AUC, i.v.} / AUC_{i.v.})^2 \). The \( C_{\text{max}} \) was obtained from the concentration versus time data.

The two-sided Student t test was used for statistical analyses.

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 \( (P < 0.001; \text{Table 1}) \) when GF120918 was given p.o. 10–20 min before the oral administration of paclitaxel. The \( C_{\text{max}} \) 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 \( C_{\text{max}} \) 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 Cl 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.
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 does not notably interact with other pathways involved in paclitaxel uptake or elimination. After both oral and i.v. paclitaxel administration, GF120918 did not change the course of the plasma concentration versus time curves of paclitaxel in knockout mice.

The results with GF120918 contrast with previous studies in mice using the P-glycoprotein-blockers cyclosporin A or PSC833 (14, 15). Although the interaction of these P-glycoprotein inhibitors have not been tested in knockout mice, the AUC of paclitaxel given in combination with oral cyclosporin A or PSC833 to wild-type mice was 2- to 5-fold higher than the AUC in knockout mice receiving paclitaxel alone. Consequently, when given p.o., the gain in the plasma AUC of paclitaxel with these cyclosporin analogues resulted for a substantial part from decreased metabolic elimination [e.g., by CYP450–3A4 (12, 13, 28)] and/or by blocking other transporters of paclitaxel. The absence of an interaction with the pharmacokinetics of paclitaxel in knockout mice does not imply that GF120918 is unable to inhibit other transporters or enzymes. In fact, it has recently been shown that this compound can also block the ABC half-transporter Breast Cancer Resistance Protein (29, 30); however, this goes without consequences because paclitaxel is not substrate of this transporter.

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)

| Group     | GF120918 (Oral) Paclitaxel dose/route (mg·kg⁻¹·h⁻¹) | AUC (ng·ml⁻¹·h) | F (%) | $C_{max}$ (ng·ml⁻¹) | CI (liter·h⁻¹·kg⁻¹) | $t_{1/2}$ (h) |
|-----------|-----------------------------------------------|-----------------|-------|-------------------|-------------------|----------------
| Wild-type |                                               |                 |       |                   |                   |              |
| A         | Vehicle 10/p.o.                                | 408 ± 36        | 8.5 ± 0.8 | 118 ± 20          | 1.95 ± 0.64       |              |
| B         | Drug 10/p.o.                                   | 2650 ± 145⁵     | 40.2 ± 1.9⁶ | 770 ± 120         | 1.98 ± 0.09       |              |
| C         | Vehicle 10/i.v.                                | 4783 ± 424      | 2.09 ± 0.19 | 1.18 ± 0.05       | 2.19 ± 0.19       |              |
| D         | Drug 10/i.v.                                   | 6600 ± 360⁶     | 1.52 ± 0.07 | 2.19 ± 0.19       |                   |              |
| Knockout  |                                               |                 |       |                   |                   |              |
| E         | Vehicle 10/p.o.                                | 2057 ± 158      | 36.1 ± 2.6 | 553 ± 130         | 2.59 ± 0.23       |              |
| F         | Drug 10/p.o.                                   | 2252 ± 112⁴     | 35.4 ± 2.4 | 530 ± 110         | 2.69 ± 0.14       |              |
| G         | Vehicle 10/i.v.                                | 5701 ± 402      | 1.73 ± 0.12 | 2.00 ± 0.11       |                   |              |
| H         | Drug 10/i.v.                                   | 6361 ± 437      | 1.57 ± 0.11 | 1.95 ± 0.11       |                   |              |

* $P < 0.001$ (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.

ACKNOWLEDGMENTS

The authors thank Ton Schrauwers for excellent biotechnical assistance.

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