**Enhanced Oral Absorption and Decreased Elimination of Paclitaxel in Mice Cotreated with Cyclosporin A**

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Abstract

Recent experiments in mice have demonstrated that the systemic exposure to p.o. administered paclitaxel is significantly enhanced by coadministration of the P-glycoprotein blocker SDZ PSC 833 (J. van Asperen et al., Br. J. Cancer, 76: 1181–1183, 1997). To facilitate further research on the feasibility of a clinically effective oral formulation of paclitaxel, it is important to know whether cotreatment with a commonly applied and commercially available P-glycoprotein blocker, e.g., cyclosporin A, has a similar effect. Here, we present a detailed study about the effects of cyclosporin A on the pharmacokinetics of p.o. and i.v. administered paclitaxel. Female FVB mice received a combined treatment of 5 or 10 mg/kg paclitaxel (either i.v. or p.o.) plus 0, 10, or 50 mg/kg cyclosporin A (p.o.). The plasma concentrations of paclitaxel were determined at several time points after drug administration using high-performance liquid chromatography. Calculated relative to the area under the plasma concentration-time curve of i.v. administered paclitaxel in mice treated without cyclosporin A, the oral bioavailability of paclitaxel increased from 9.3% up to 67% with coadministration of cyclosporin A. The bioavailability in mice co-treated with 10 or 50 mg/kg cyclosporin A appeared to be similar. The effect of cyclosporin A on the systemic exposure to p.o. administered paclitaxel was the result of both a significantly decreased clearance and an increased uptake. A histological examination revealed that the enhanced absorption was not caused by gastrointestinal toxicity. We conclude that cyclosporin A and SDZ PSC 833 are equally effective in increasing the systemic exposure to p.o. administered paclitaxel. These data are promising for the development of a clinically useful oral formulation of this cytostatic drug and indicate that cyclosporin A is a suitable agent for further research of this concept.

Introduction

Paclitaxel is a taxane drug with considerable activity against a wide variety of human neoplastic disorders. It is usually administered by i.v. infusion (1, 2). The i.v. formulation contains Cremophor EL, a vehicle that has been associated with severe hypersensitivity reactions (3, 4). These adverse effects may be prevented by oral administration of paclitaxel, when Cremophor EL can be omitted from the oral formulation or is not absorbed from the gut. Furthermore, oral administration is, in general, also preferred over i.v. chemotherapy by patients (5). However, the low oral bioavailability of paclitaxel observed in preclinical studies has discouraged the development of an oral formulation (6). Recent experiments in mice, however, have demonstrated that intestinal P-glycoprotein plays an important role in the limited absorption of p.o. administered paclitaxel (7). P-glycoprotein is a large plasma membrane protein that was initially discovered because of its ability to confer multidrug resistance to mammalian tumor cells (8). Subsequent studies demonstrated that this protein is also present in many normal tissues (9). In the intestine, P-glycoprotein is almost exclusively located in the apical membrane of mature enterocytes (10–12), where it mediates the transport of substrates out of the cell into the intestinal lumen, thereby forming a barrier to drug absorption. Among its substrates are a wide variety of clinically important drugs, such as antineoplastic agents (e.g., anthracyclines, Vinca alkaloids, and taxanes), cardiac drugs (e.g., verapamil and digoxin), antifungal agents (e.g., ketoconazole), and immunosuppressive agents (e.g., cyclosporin A and tacrolimus). Reversal agents, compounds that block or inhibit P-glycoprotein, are currently under clinical investigation in an attempt to overcome P-glycoprotein-mediated multidrug resistance of tumors. In line with the role of P-glycoprotein in the oral absorption of paclitaxel, it was hypothesized that concomitant oral administration of a reversal agent might also increase the oral bioavailability of paclitaxel. Experiments in mice confirmed this hypothesis. A substantially increased AUCoral2 of paclitaxel was observed with concomitant administration of the reversal agent and cyclosporine D analogue SDZ PSC 833 (13). In addition, the data indicated that SDZ PSC 833 not only increased the AUCoral of paclitaxel by enhancing its absorption from the intestinal lumen but also, at least partially, by decreasing its elimination. The promising results of this study suggested that this concept may lead to the development of a clinically useful oral formulation of paclitaxel. However, large-scale investigations with SDZ PSC 833 are complicated, because it is not yet a licensed

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2 The abbreviations used are: AUC, area under the plasma concentration versus time curve; CI, clearance.
drug. Therefore, we have currently investigated whether similar results can be obtained in mice with another widely applied and commercially available reversal agent, cyclosporin A. Furthermore, the design of these experiments permitted a differentiation between the effects of the reversal agent on the oral absorption of paclitaxel and those on its elimination.

### Materials and Methods

**Animals.** All experiments were performed with female FVB wild-type mice between 9 and 16 weeks of age. They were housed and handled according to institutional guidelines. Food (Hope Farms B.V., Woerden, the Netherlands) and acidified water were given ad libitum.

**Drugs and Chemicals.** A sterile 6 mg/ml solution of paclitaxel in Cremophor EL-ethanol (1:1, v/v; Taxol) and 2'-methylpaclitaxel were obtained from Bristol-Myers Squibb (Princeton, NJ). Paclitaxel (solid) originated from Sankyo Co. Ltd. (Tokyo, Japan). A clinical formulation of 50 mg/ml cyclosporin A in Cremophor EL-ethanol (67:1:32.9, v/v; Sandimmune) was obtained from Sandoz (Basel, Switzerland). Cremophor EL and polysorbate 80 (Tween 80) were purchased from Sigma Chemical Co. (St. Louis, MO) and Brocacef B.V. (Maarssen, the Netherlands), respectively. Sterile saline was supplied by NPBI (Emmer-Compascuum, the Netherlands). All other chemicals were of analytical or Lichrosolv gradient grade and originated from E. Merck (Darmstadt, Germany). Water was purified by the Milli-Q Plus system (Millipore, Milford, MA). Blank human plasma was obtained from healthy donors.

**Drug Formulations.** Eight oral formulations were prepared, each containing standardized amounts of Cremophor EL, ethanol, and saline. The compositions of these formulations are given in Table 1. By dissolving 6 mg of paclitaxel (solid) per ml of ethanol-polysorbate 80 (1:1, v/v) a stock solution for the preparation of two i.v. formulations was obtained. The stock solution was diluted with appropriate amounts of ethanol, polysorbate 80, and saline resulting in formulations containing either 1.5 or 0.75 mg/ml paclitaxel for administration of dose levels of 10 or 5 mg/kg, respectively. Both i.v. formulations contained standardized amounts of polysorbate 80, ethanol, and saline (1:1:5, v/v/v).

**Study Design.** For the oral treatment with paclitaxel, 10 μl per g body weight of formulation A, B, C, D, or E (see Table 1) was administered by gavage under light diethyl ether anesthesia. Blood samples were collected at 1, 2, 4, and 8 h after drug administration. Additional blood samples were taken at 12 and 16 h after drug administration in groups treated with formulation B, D, or E. For groups that were to receive paclitaxel i.v., 10 μl per g body weight of formulation F, G, or H was p.o. administered according to the procedure described above. Subsequently, at 30 min after administration of the oral formulation, 6.67 μl per g body weight of an i.v. formulation of paclitaxel were injected into a tail vein under light diethyl ether anesthesia. Blood samples were collected at 5 and 30 min and at 1, 2, 4, and 8 h after administration of paclitaxel. Additional blood samples were obtained from animals treated with formulation G or H, at 12 and 16 h and for one group also at 24 h after i.v. administration.

Two to six animals were used per time point in all experiments. Blood was obtained from the retro-orbital plexus under diethyl ether anesthesia and collected in heparinized tubes. The plasma fraction was separated by centrifugation (10 min, 2000 × g, 4°C) and stored at −20°C until analysis. Immediately after the withdrawal of blood, the animals were sacrificed by cervical dislocation. Animals receiving 10 mg/kg paclitaxel (i.v. or p.o.) with or without cyclosporin A and animals receiving 5 mg/kg paclitaxel (i.v. or p.o.) in combination with 50 mg/kg cyclosporin A were also used for histological examination. For this purpose, several tissues of groups of four mice sacrificed at −12 h after paclitaxel administration were dissected. These tissues included: brain, heart, lung, spleen, liver, kidney, stomach, duodenum, jejunum, ileum, cecum, colon, peripheral and mesenteric lymph nodes, thymus, and adrenal. They were collected in vials containing Harrison’s fixative (14). Furthermore, in three additional groups, each consisting of two mice, the toxicity of the paclitaxel plus cyclosporin A regimen was checked until 16 days after drug administration. They received either 10 mg/kg paclitaxel (i.v. or p.o.) or 5 mg/kg paclitaxel (i.v.). All three groups were concomitantly treated with 50 mg/kg cyclosporin A.

### Table 1 Composition of the oral formulations used for each treatment group

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Paclitaxel (mg/kg)</th>
<th>Cyclosporin A (mg/kg)</th>
<th>Taxol (μl)</th>
<th>Sandimmune (μl)</th>
<th>Cremophor EL (μl)</th>
<th>Ethanol (μl)</th>
<th>Saline (μl)</th>
<th>Final concentration (mg/ml)</th>
<th>Cyclosporin A (mg/ml)</th>
<th>Cremophor EL (%)</th>
<th>Ethanol (%)</th>
<th>Saline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5 (p.o.)</td>
<td>0</td>
<td>833</td>
<td>1084</td>
<td>750</td>
<td>7333</td>
<td>1.0</td>
<td>0.5</td>
<td>0.0</td>
<td>15.0</td>
<td>11.7</td>
<td>73.3</td>
</tr>
<tr>
<td>B</td>
<td>5 (p.o.)</td>
<td>50</td>
<td>833</td>
<td>417</td>
<td>7333</td>
<td>7333</td>
<td>1.0</td>
<td>0.5</td>
<td>0.0</td>
<td>15.0</td>
<td>11.7</td>
<td>73.3</td>
</tr>
<tr>
<td>C</td>
<td>10 (p.o.)</td>
<td>0</td>
<td>1667</td>
<td>667</td>
<td>333</td>
<td>7333</td>
<td>1.0</td>
<td>1.0</td>
<td>0.0</td>
<td>15.0</td>
<td>11.7</td>
<td>73.3</td>
</tr>
<tr>
<td>D</td>
<td>10 (p.o.)</td>
<td>10</td>
<td>1667</td>
<td>534</td>
<td>266</td>
<td>7333</td>
<td>1.0</td>
<td>1.0</td>
<td>0.0</td>
<td>15.0</td>
<td>11.7</td>
<td>73.3</td>
</tr>
<tr>
<td>E</td>
<td>10 (p.o.)</td>
<td>50</td>
<td>1667</td>
<td>0</td>
<td>0</td>
<td>7333</td>
<td>1.0</td>
<td>1.0</td>
<td>0.0</td>
<td>15.0</td>
<td>11.7</td>
<td>73.3</td>
</tr>
<tr>
<td>F</td>
<td>5/10 (i.v.)</td>
<td>0</td>
<td>0</td>
<td>1500</td>
<td>1167</td>
<td>7333</td>
<td>0</td>
<td>1.0</td>
<td>0.0</td>
<td>15.0</td>
<td>11.7</td>
<td>73.3</td>
</tr>
<tr>
<td>G</td>
<td>10 (i.v.)</td>
<td>0</td>
<td>0</td>
<td>200</td>
<td>1367</td>
<td>7333</td>
<td>0</td>
<td>1.0</td>
<td>0.0</td>
<td>15.0</td>
<td>11.7</td>
<td>73.3</td>
</tr>
<tr>
<td>H</td>
<td>5/10 (i.v.)</td>
<td>50</td>
<td>0</td>
<td>1000</td>
<td>834</td>
<td>7333</td>
<td>0</td>
<td>5.0</td>
<td>0.0</td>
<td>15.0</td>
<td>11.7</td>
<td>73.3</td>
</tr>
</tbody>
</table>

*CsA, cyclosporin A; CrEL, Cremophor EL.

*Taxol, a solution of 6 mg/ml paclitaxel in Cremophor EL-ethanol (1:1, v/v).

*Sandimmune, a formulation containing 50 mg/ml cyclosporin A in Cremophor EL-ethanol (67:1:32.9, v/v).

*Groups that were to receive either 5 or 10 mg/kg paclitaxel i.v.
Fig. 1 Plasma concentration versus time curves of paclitaxel in female FVB mice after i.v. (A and B) or oral (C and D) administration of 5 mg/kg (A and C) or 10 mg/kg (B and D) paclitaxel. The animals were cotreated with oral dose levels of 0 mg/kg (□ and □), 10 mg/kg (●), or 50 mg/kg (● and □) cyclosporin A. Data points, means; bars, SE. The absence of error bars indicates that the standard error is smaller than the size of the symbols. Two to six animals were used per time point.

**Drug Analysis.** The analysis of paclitaxel was performed as described in detail previously (15). In summary, paclitaxel was extracted from plasma by a double liquid-liquid extraction using diethyl ether, followed by a solid-phase extraction. After evaporation of the solvent, the residue was reconstituted in a mixture of acetonitrile-methanol-water and subjected to reversed-phase high-performance liquid chromatography with UV detection.

**Histology.** Tissue samples were routinely fixed in Har- rison’s fixative (14) and processed for microscopy. The 5-μm sections were stained with H&E.

**Pharmacokinetics.** Pharmacokinetic parameters were calculated by noncompartmental methods using the software package Quattro Pro for Windows (Version 5.0, 1993; Borland International, Scotts Valley, CA). The AUC\textsubscript{i.v.} and the AUC\textsubscript{oral} of paclitaxel were calculated by the linear trapezoidal rule, without extrapolation to infinity. The SE of the AUC was calculated with the law of propagation of errors (16). To calculate Cl, the following formula was used: (1)

\[ Cl = \text{dose}/\text{AUC}_{i.v.} \]

The elimination rate constant, \( k \), and its SE were calculated by linear regression analysis of the ln(concentration) versus time data points of the final part of the plasma concentration-time curve of i.v. administered paclitaxel. The terminal half-life, \( t_{1/2\text{IV}} \), was calculated using the formula: (2)

\[ t_{1/2\text{IV}} = \ln 2/k \]

\( F \) (oral bioavailability) was calculated with the formula: (3)

\[ F = (\text{AUC}_{oral}/\text{AUC}_{i.v.}) \times 100\% \]

**Statistics.** The unpaired Student’s \( t \) test (two-tailed) was used to compare the pharmacokinetic parameters. A \( P \) < 0.05 was regarded as statistically significant.

**Results**

The coadministration of cyclosporin A had a striking effect on the plasma concentration-time curves of both i.v. and p.o. administered paclitaxel (Fig. 1). Treatment with 50 mg/kg cyclosporin A resulted in 2.6- and 3.3-fold higher AUC\textsubscript{i.v.} s of paclitaxel at dose levels of 5 and 10 mg/kg, respectively (Table 2). A 1.8-fold higher AUC\textsubscript{oral} was observed with coadministration of 10 mg/kg cyclosporin A. Administration of cyclosporin A did not or only slightly change the \( c_{\text{max}} \) (maximum plasma levels) of i.v. administered paclitaxel, whereas \( t_{1/2\text{IV}} \) was significantly prolonged. The AUC\textsubscript{oral} of paclitaxel increased by a factor of 7.4–13.4 with coadministration of cyclosporin A.
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Table 2  Pharmacokinetic parameters of paclitaxel after i.v. or p.o. administration of 5 or 10 mg/kg to mice cotreated with oral dose levels of 0, 10, or 50 mg/kg of cyclosporin A.

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Parameter</th>
<th>0/5</th>
<th>50/5</th>
<th>0/10</th>
<th>10/10</th>
<th>50/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v.</td>
<td>AUC_{iv} (mg · h/liter)</td>
<td>4.48 ± 0.21</td>
<td>11.72 ± 0.39</td>
<td>10.81 ± 0.87</td>
<td>19.5 ± 1.01</td>
<td>35.93 ± 1.24</td>
</tr>
<tr>
<td></td>
<td>CI (liters/h/kg)</td>
<td>1.12 ± 0.05</td>
<td>0.43 ± 0.01</td>
<td>0.92 ± 0.07</td>
<td>0.51 ± 0.03</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>t_{1/2,iv} (h)</td>
<td>2.15 ± 0.08</td>
<td>5.45 ± 0.22</td>
<td>2.37 ± 0.22</td>
<td>3.03 ± 0.16</td>
<td>3.79 ± 0.19</td>
</tr>
<tr>
<td>Oral</td>
<td>CL_{max} (μg/ml)</td>
<td>5.26 ± 0.43</td>
<td>6.54 ± 0.22</td>
<td>11.05 ± 2.36</td>
<td>13.40 ± 1.58</td>
<td>16.50 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>AUC_{oral} (mg · h/liter)</td>
<td>0.41 ± 0.03</td>
<td>3.02 ± 0.19</td>
<td>0.50 ± 0.07</td>
<td>4.55 ± 0.51</td>
<td>6.68 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>e_{max} (μg/ml)</td>
<td>0.09 ± 0.02</td>
<td>0.31 ± 0.04</td>
<td>0.08 ± 0.02</td>
<td>0.64 ± 0.13</td>
<td>0.73 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>F (%)</td>
<td>9.26 ± 0.55</td>
<td>25.79 ± 1.25</td>
<td>4.60 ± 0.52</td>
<td>23.30 ± 1.91</td>
<td>21.20 ± 1.11</td>
</tr>
</tbody>
</table>

*P < 0.05, as compared to mice similarly treated with paclitaxel but without cyclosporin A.

Due to the limited number of time points, these values must be regarded as estimations.

which resulted in a 2.8–5.1-fold higher oral bioavailability. The oral bioavailability of paclitaxel appeared to be similar at dose levels of 10 and 50 mg/kg cyclosporin A. In groups treated with paclitaxel alone, a 2-fold lower oral bioavailability was observed at 10 mg/kg, as compared to 5 mg/kg. A histological examination was performed to exclude the possibility that the increased absorption of paclitaxel in mice cotreated with cyclosporin A was a consequence of severe intestinal toxicity and to detect potential acute adverse effects of the regimen. Symptoms of toxicity were not observed in any of the tissues examined. Hence, the increased oral bioavailability of paclitaxel did not result from tissue damage. In addition, loss of body weight or other macroscopic signs of toxicity were not observed in animals monitored up to 16 days after a single treatment with paclitaxel plus 50 mg/kg cyclosporin A.

Discussion

These results show that cotreatment with cyclosporin A significantly increases the oral bioavailability of paclitaxel. Moreover, the AUC_{oral} of paclitaxel in mice cotreated with 50 mg/kg cyclosporin A is comparable to that previously observed in mice cotreated with 50 mg/kg SDZ PSC 833 (13). Here, we demonstrate that the increase in AUC_{oral} is not only caused by an enhanced absorption but also by a decreased elimination. One of the factors that probably contributes to the enhanced absorption of paclitaxel is inhibition of intestinal P-glycoprotein by cyclosporin A. We have reported previously that the oral bioavailability of paclitaxel in mdrla(-/-) mice, which completely lack intestinal P-glycoprotein, was -35% (7). In that study, the AUC_{iv} of paclitaxel in wild-type (normal) mice was lower than that presently observed in animals receiving a comparable treatment with paclitaxel alone, probably due to biological variation and limited sampling intervals. Relative to this interexperimental variation, the oral bioavailability of paclitaxel in mice cotreated with cyclosporin A, which averaged 23.4%, approaches that in mdrla(-/-) mice. Another important factor that may contribute to the increased uptake of paclitaxel in mice treated with cyclosporin A is metabolic competition. Both paclitaxel and cyclosporin A are substrates for the cytochrome P450 3A4 isoforms (17, 18), which are found in the liver as well as in the enterocytes. Hence, coadministration of cyclosporin A may reduce the first-pass metabolic elimination of paclitaxel in the gut wall and the liver. In addition, inhibition of outward directed transport mechanisms other than P-glycoprotein and an increased stability in the gastrointestinal tract may also play a role in the enhanced absorption. The reduced CI of paclitaxel in animals treated with cyclosporin A may have several causes. The direct secretion via the gut wall may be reduced due to an inhibition of P-glycoprotein in the intestines. There may be a diminished hepatic CI as a result of metabolic competition because, as already discussed above, paclitaxel and cyclosporin A are both metabolized by cytochrome P450 3A4. Other potential causes may include the occurrence of cholestasis (19) and the inhibition of transport mechanisms other than P-glycoprotein (20, 21).

Cremophor EL, a vehicle that is used in the clinical formulation of paclitaxel, has been shown to cause nonlinear pharmacokinetics of i.v. administered paclitaxel in mice (22). Therefore, we have used an i.v. formulation that does not contain this additive. Because the systemic uptake of Cremophor EL from the gastrointestinal tract is very low, the oral formulations could be prepared with the clinical formulation of paclitaxel. Moreover, a 2-fold higher AUC_{iv} was observed in mice receiving 10 mg/kg paclitaxel without cyclosporin A, compared with mice receiving 5 mg/kg paclitaxel without cyclosporin A (Table 2), indicating linear pharmacokinetics.

In conclusion, cyclosporin A and SDZ PSC 833 are equally effective in increasing the systemic exposure to p.o. administered paclitaxel. The oral bioavailability of paclitaxel in mice treated without cyclosporin A was 4.6–9.3%. Calculated relative to the AUC_{iv} of paclitaxel in these mice, the oral bioavailability increased up to 67% with coadministration of cyclosporin A. This suggests that a high systemic exposure can be obtained with clinical applicability of a suitable oral formulation of paclitaxel. In addition, oral administration of paclitaxel did not generate gastrointestinal toxicity. Dose levels of 10 and 50 mg/kg cyclosporin A seemed to be equally potent in enhancing the absorption of paclitaxel, but the latter had a more pronounced effect on drug elimination, resulting in a higher AU_{Coral}. In humans, chronic oral administration of 10 mg/kg/day...
cyclosporin A, which, in general, results in plasma levels in the range of 200 ng/ml, is well tolerated (23). These data indicate that cyclosporin A may be used to examine the feasibility of a clinically effective oral formulation of paclitaxel.

References


Enhanced oral absorption and decreased elimination of paclitaxel in mice cotreated with cyclosporin A.

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