Cancer Chemother Pharmacol (2004) 53: 173–178
DOI 10.1007/s00280-003-0720-y

ORIGINAL ARTICLE

E. Marleen Kemper · Cindy Cleypool · Willem Boogerd
Jos H. Beijnen · Olaf van Tellingen

The influence of the P-glycoprotein inhibitor zosuquidar trihydrochloride (LY335979) on the brain penetration of paclitaxel in mice

Received: 19 June 2003 / Accepted: 14 September 2003 / Published online: 7 November 2003

Abstract We determined the effect of zosuquidar·3HCl, an inhibitor of P-gp, on the penetration of the anticancer drug paclitaxel into the brain. Zosuquidar·3HCl was administered orally at 25 and 80 mg/kg 1 h before i.v. paclitaxel and i.v. at 20 mg/kg 10 min and 1 h before paclitaxel. The concentrations of paclitaxel in plasma and tissues and of zosuquidar·3HCl in plasma were quantified by high-performance liquid chromatography. The results revealed 3.5-fold and 5-fold higher paclitaxel levels in the brain of wild-type mice treated orally with 25 and 80 mg/kg zosuquidar·3HCl, respectively. However, complete inhibition as in P-gp knockout mice (11-fold increase) was not achieved. Zosuquidar·3HCl also increased the paclitaxel concentrations in plasma and tissues to levels similar to those observed in P-gp knockout mice, suggesting selective P-gp inhibition of zosuquidar·3HCl. When zosuquidar·3HCl was administered i.v. 10 min before paclitaxel, the paclitaxel levels in the brain of wild-type mice increased by 5.6-fold, whereas the increase was only 2.1-fold when zosuquidar·3HCl was administered 1 h before paclitaxel. This suggests that the inhibition of P-gp at the blood-brain barrier by zosuquidar·3HCl is rapidly reversible and that the concentrations of zosuquidar·3HCl in the plasma have already declined to levels insufficient to inhibit P-gp at the blood-brain barrier. In conclusion, zosuquidar·3HCl is only moderately active as an inhibitor of P-gp at the blood-brain barrier.

Keywords Zosuquidar trihydrochloride · Paclitaxel · Blood-brain barrier · P-glycoprotein

E. M. Kemper · C. Cleypool · O. van Tellingen (✉)
Department of Clinical Chemistry,
The Netherlands Cancer Institute/Antoni van Leeuwenhoek Huis,
Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
E-mail: otel@nki.nl
Tel.: +31-20-5122792
Fax: +31-20-6172625

W. Boogerd
Department of Neurology, Slotervaart Hospital,
Louwesweg 6, 1066 EC Amsterdam, The Netherlands

J. H. Beijnen · W. Boogerd
Department of Medical Oncology,
The Netherlands Cancer Institute/Antoni van Leeuwenhoek Huis,
Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

J. H. Beijnen
Department of Pharmacy and Pharmacology,
The Netherlands Cancer Institute/Slotervaart Hospital,
Louwesweg 6, 1066 EC Amsterdam, The Netherlands

J. H. Beijnen
Division of Drug Toxicology,
Faculty of Pharmaceutical Sciences, Utrecht University,
Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands

Introduction

In anticancer therapy the brain is often regarded as a "sanctuary site" for cytotoxic drugs due to their inability to cross the blood-brain barrier. Studies in P-glycoprotein (P-gp) knockout mice have demonstrated that the poor uptake of several anticancer drugs into the brain is caused by the presence of P-gp in the blood-brain barrier [13, 14].

In a previous study we have shown the potential usefulness of P-gp inhibitors for increasing the access of the potent anticancer drug paclitaxel into the brain [9]. The P-gp inhibitors valspodar (PSC833) and elacridar (GF120918) were able to increase the concentration of paclitaxel in the brain substantially. However, a complete inhibition of P-gp as obtained in the P-gp knockout mouse model was not achieved. The best result was achieved with elacridar. The levels of paclitaxel in the brain ranged to 80–90% of those achieved in P-gp knockout mice. Valspodar was similarly effective, but also markedly reduced the systemic clearance of paclitaxel, rendering dose reductions of paclitaxel mandatory when combined with valspodar to prevent an increase in paclitaxel-related side effects. These dose reductions would in their turn reduce the amount of paclitaxel that reaches the brain.

Recently, several studies with the experimental P-gp inhibitor zosuquidar·3HCl have been published (reviewed by Dantzig et al. [6]). In vitro studies with zosuquidar·3HCl have shown an increased sensitivity of multidrug-resistant cell lines to the anticancer drugs doxorubicin, vinblastine, etoposide and paclitaxel [4, 16]. Studies with tumor-bearing animals have shown a prolonged survival in murine leukemia models that overexpress P-gp [4, 19] and have shown growth reductions in a subcutaneously implanted non-small-cell lung carcinoma xenograft model in combination with paclitaxel [4]. Moreover, in contrast to many of the first- and second-generation P-gp inhibitors, zosuquidar·3HCl has shown minimal or no interaction with the pharmacokinetics of chemotherapeutic agents [19]. Zosuquidar·3HCl appears to possess a very low affinity for enzymes of the cytochrome P-450 family and for the drug-transporting proteins MRP1, MRP2 and BCRP [5, 15]. As a consequence it was anticipated that zosuquidar·3HCl could be combined with anticancer drugs given at full doses to patients without increasing toxicity. This has recently been confirmed in a clinical study using doxorubicin [12].

These previous studies suggest that zosuquidar·3HCl is a potent and selective inhibitor of P-gp. The efficacy of zosuquidar·3HCl, however, in increasing the penetration of substrate anticancer drugs into the brain by inhibition of P-gp has not yet been studied. To address this issue we determined the effect of zosuquidar·3HCl on the penetration of paclitaxel into the brain. We measured paclitaxel concentrations in brain tissue, in plasma and in other tissues to determine the effect of coadministration of zosuquidar·3HCl on the pharmacokinetics of paclitaxel. P-gp knockout mice were used as a model for complete inhibition of P-gp. We also determined the pharmacokinetics of zosuquidar·3HCl in plasma of mice after i.v. and oral administration.

Materials and methods

Drug solutions

Zosuquidar·3HCL (lot no. 172SB9) was kindly provided by Eli Lilly (Indianapolis, Ind.). A stock solution of 5 mg/ml of zosuquidar·3HCL in vehicle solution was prepared fresh on the day of administration. The vehicle solution consisted of 20 g/l mannitol and 1.5 g/l of glycine (both from Merck, Darmstadt, Germany) in water for injection (Braun, Emmer-Compascuum, The Netherlands) and adjusted to a pH of 2.7 with hydrochloric acid. Further dilutions were made in sterile saline (Braun).

A stock solution of 6 mg/ml paclitaxel (Sankyo, Tokyo, Japan) was made in ethanol (Merck) and Tween 80 (Sigma Chemicals Co, St. Louis, Mo.) (1:1 v/v). This stock solution was diluted further with sterile saline to a final concentration of 1.5 mg/ml.

Animals

Female FVB wild-type and P-gp knockout [*mdr1ab*(−/−)] mice at 10–14 weeks of age were housed and handled in accordance with Dutch national law. The animals were provided with food (Hope Farms, Woerden, The Netherlands) and acidified water ad libitum. All experiments were approved by the local committee for animal experiments.]

Study design

All mice in this study received 10 mg/kg paclitaxel by i.v. bolus injection into the tail vain. The study comprised six different study groups:

1. Wild-type control mice receiving paclitaxel alone
2. Wild-type mice receiving 25 mg/kg zosuquidar·3HCl orally 1 h before paclitaxel
3. Wild-type mice receiving 80 mg/kg zosuquidar·3HCl orally 1 h before paclitaxel
4. Wild-type mice receiving 20 mg/kg zosuquidar·3HCl i.v. 10 min before paclitaxel
5. Wild-type mice receiving 20 mg/kg zosuquidar·3HCl i.v. 1 h before paclitaxel
6. P-gp knockout control mice receiving paclitaxel alone

Sampling was performed at 1, 4, 8 and 24 h. Four to five mice were used per time-point per group. Blood was obtained by cardiac puncture under anesthesia with methoxyflurane (Medical Developments, Australia, Melbourne, Australia). Animals were killed by cervical dislocation, and the brain, liver, kidneys, lungs and heart were collected. Plasma samples were obtained by centrifugation (10 min, 3000 *g*) and stored at −20°C until analysis. The tissues were homogenized in 4% (w/v) bovine serum albumin (Roche Diagnostics, Mannheim, Germany) in water (0.1–0.2 g/ml) and stored at −20°C until analysis.

For the determination of the pharmacokinetics of zosuquidar·3HCl in plasma, FVB wild-type mice were treated orally with 25 or 80 mg/kg zosuquidar·3HCl or i.v. with 20 mg/kg zosuquidar·3HCl. Sampling was performed at 30 min and 1, 2, 4, 8 and 24 h, and for the i.v. dose also at 10 min. This pharmacokinetic study was performed in a separate set of animals.

Analytical methods

All chemicals used for drug analysis were of analytical or Lichrosolv gradient grade and were purchased from Merck (Darmstadt, Germany). Paclitaxel was determined with a validated HPLC method with UV detection as described previously [17]. Paclitaxel was extracted from plasma and tissue homogenates by a double liquid-liquid extraction with diethyl ether followed by a solid-phase extraction. The lower and upper limits of quantitation using 200 µl sample were, respectively, 25 and 5000 ng/ml. Extraction recoveries ranged from 76% to 85% for all materials.

Zosuquidar·3HCl in mouse plasma was determined using an HPLC method with fluorescence detection [8]. In summary, using 50 µl sample, zosuquidar·3HCl was extracted from plasma with *t*-butyl methyl ether. Chlorpromazine was used as internal standard. Separation was performed using a 2.1×150 mm column packed with 3.5 µm Symmetry C-18 material (Waters, Milford, Mass.) and a mobile phase of 38% (v/v) acetonitrile in 50 m*M* ammonium acetate buffer, pH 3.8, containing 0.005 *M* 1-octyl sulfonic acid, which was delivered at 0.2 ml/min. The fluorescence detector was set at an excitation wavelength of 260 nm and an emission wavelength of 460 nm. The method was fully validated, and the lower and upper limits of quantitation for mouse plasma were 20 and 1000 ng/ml.

Pharmacokinetic and statistical calculations

The plasma area under the curve (AUC) of paclitaxel and the AUC of paclitaxel in the tissues were calculated by the linear trapezoidal rule from time 0 to the last time-point at which the

concentration was above the lower limit of quantitation (LLQ) using the formula:

$$AUC = \sum_{i=2}^{n} concentration_i \cdot \frac{(\Delta time_{i-1} + \Delta time_i)}{2}$$

The standard error (SE) of the AUC was calculated with the law of propagation of errors using the formula:

$$SE_{AUC} = \sqrt{\left(\sum_{i=2}^{n} SE_i \cdot \frac{(\Delta time_{i-1} + \Delta time_i)}{4}\right)}$$

The two-sided Student *t* test was used for statistical analyses. *P* values < 0.05 were regarded as statistically significant.

The pharmacokinetic parameters of zosuquidar·3HCl after i.v. administration were calculated using the program MW/Pharm (Mediware, Groningen, The Netherlands) [11]. The oral bioavailability of zosuquidar·3HCl was calculated on AUC values using the formula $AUC_{oral}/AUC_{i.v.} \times 100\%$.

Results

The concentrations of paclitaxel in brain tissue of wild-type mice were low, and increased after coadministration of the P-gp inhibitor zosuquidar·3HCl (Fig. 1). An oral dose of 25 mg/kg of zosuquidar·3HCl increased the brain concentrations by about 2.5-fold at 1 h and 5-fold at 24 h after paclitaxel administration. Overall there was a 3.5-fold increase in the $AUC_{brain,0–24\ h}$ ($P < 0.001$; Table 1).

Increasing the dose of zosuquidar·3HCl to 80 mg/kg did not further increase the paclitaxel concentrations in brain tissue at 1 and 4 h after drug administration (Fig. 1), but did result in significantly higher brain concentrations of paclitaxel at 8 and 24 h ($P < 0.01$). At 48 h the brain levels of paclitaxel were still 299 ± 17 ng/g (mean ± SE), which is similar to the concentrations observed at the earlier time-points. Overall, increasing the dose of zosuquidar·3HCl further increased the $AUC_{brain,0–24\ h}$ of paclitaxel 5.2-fold relative to the value in the wild-type control mice ($P < 0.001$; Table 1). However, this value was only 45% of the $AUC_{brain,0–24\ h}$ achieved in P-gp knockout mice. This substantial difference between wild-type mice receiving paclitaxel with zosuquidar·3HCl and P-gp knockout mice resulted from the fact that only in the latter group did the brain concentration increase further between 1 and 4 h after drug administration, while the brain concentrations in wild-type mice treated with zosuquidar·3HCl remained constant during the study period.

We also tested the efficacy of i.v. zosuquidar·3HCl in increasing paclitaxel levels in the brain. When 20 mg/kg of zosuquidar·3HCl was administered 10 min before paclitaxel the concentrations of paclitaxel in the brain were similar to those achieved with an oral dosage of 80 mg/kg ($P > 0.1$; Fig. 1). This resulted in a 5.4-fold increase in the $AUC_{brain,0–24\ h}$ (Table 1). However, when the lag-time between i.v. zosuquidar·3HCl and paclitaxel

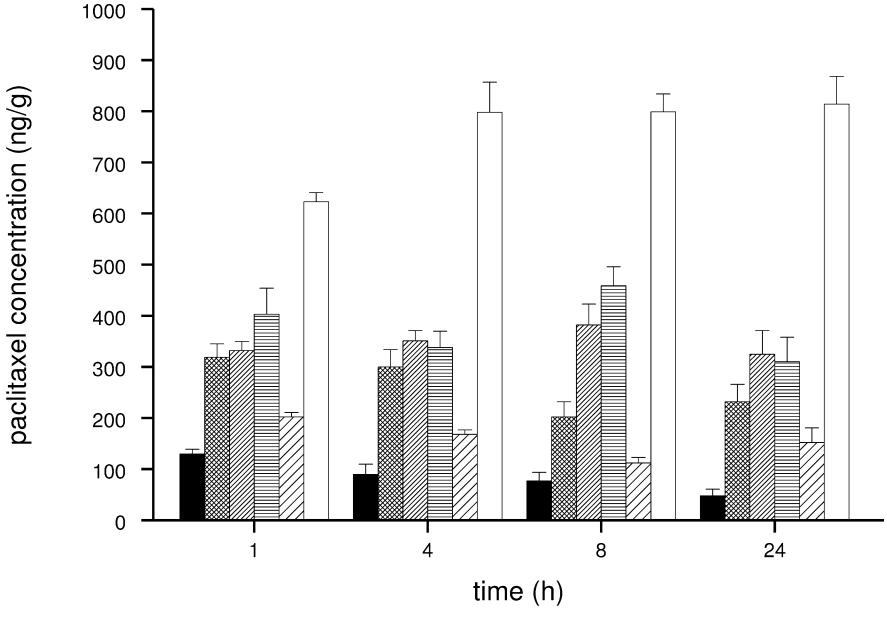

Fig. 1 Paclitaxel concentrations in the brain of wild-type mice at 1, 4, 8 and 24 h after receiving the following treatments (left to right *bars 1–5*): *bar 1* paclitaxel alone, *bars 2 and 3* paclitaxel 1 h after oral zosuquidar·3HCl at 25 mg/kg (*bar 2*) or 80 mg/kg (*bar 3*), *bars 4 and 5* paclitaxel 10 min (*bar 4*) or 1 h (*bar 5*) after i.v. zosuquidar·3HCl at 20 mg/kg. P-gp knockout mice were used as a reference for complete blockade of P-gp (*bar 6*). Paclitaxel was administered at t = 0 h

Table 1 Area under the concentration-time curves (mean ± SE) of paclitaxel in plasma (μg·h/ml) and tissues (μg·h/g) from 0 to 8 h in plasma and heart and from 0 to 24 h in the other tissues

	Plasma	Brain	Liver	Kidneys	Lungs	Heart
Wild-type control	3.2 ± 0.4	1.6 ± 0.2	126 ± 6	48.1 ± 2.3	34.9 ± 2.5	18.4 ± 1.0
25 mg/kg, oral	5.0 ± 0.1	5.6 ± 0.4	193 ± 10	50.6 ± 1.9	36.8 ± 1.4	21.8 ± 0.4
80 mg/kg, oral	5.2 ± 0.4	8.3 ± 0.6	222 ± 8	66.6 ± 2.2	47.3 ± 2.2	29.1 ± 1.2
20 mg/kg, i.v., −10 min	5.5 ± 0.3	8.6 ± 0.6	199 ± 6	68.4 ± 2.7	57.3 ± 3.3	32.9 ± 2.2
20 mg/kg, i.v., −1 h	5.1 ± 0.5	3.4 ± 0.3	244 ± 11	62.5 ± 2.2	42.3 ± 2.1	30.4 ± 2.1
P-gp knockout control	4.7 ± 0.2	18.6 ± 0.6	203 ± 10	74.5 ± 2.6	76.1 ± 2.1	35.3 ± 1.0

was extended to 1 h, the $AUC_{brain,0–24\ h}$ was much lower and only 2-fold higher relative to the value in the wild-type mice ($P < 0.001$).

Coadministration of zosuquidar·3HCl resulted in higher plasma concentrations of paclitaxel than in the wild-type mice. Irrespective of the dose and route of administration of zosuquidar·3HCl, the $AUC_{plasma,0–8\ h}$ of paclitaxel increased by about 1.6-fold (Table 1). These values were similar to those observed in the P-gp knockout mice receiving paclitaxel as a single agent ($P > 0.2$). While the plasma concentrations of paclitaxel declined to undetectable levels within 24 h of administration, the paclitaxel levels in the brain remained relatively constant during the 24-h study period.

The paclitaxel levels in the other tissues followed a pattern similar to that in the plasma (Table 1) and the increased uptake of paclitaxel by tissues can be explained by the decreased plasma clearance of paclitaxel in the presence of zosuquidar·3HCl. For example, a 1.7-fold increase in paclitaxel $AUC_{plasma,0–8\ h}$ after i.v. administration of zosuquidar·3HCl (10 min before paclitaxel) resulted in a 1.6-fold higher $AUC_{liver,0–24\ h}$, a 1.4-fold higher $AUC_{kidneys,0–24\ h}$, a 1.6-fold higher $AUC_{lungs,0–24\ h}$ and a 1.8-fold higher $AUC_{heart,0–8\ h}$. The highest paclitaxel levels were found in the liver, followed by the lungs, kidneys and the heart. In the latter, the paclitaxel levels declined to undetectable levels within 24 h in all treatment groups. With 25 mg/kg of zosuquidar·3HCl given orally, the AUC levels found in kidneys, lungs and heart were lower than expected based on the plasma levels. In none of the treatment groups did the paclitaxel tissue AUCs exceed those in the P-gp knockout control group.

We also measured the concentrations of zosuquidar·3HCl in plasma (Fig. 2). After i.v. administration, the plasma concentration-time curve followed biexponential decay kinetics with distribution and elimination half-lives of 15 min and 2.1 h, respectively. The clearance was 3.3 l/h per kg and the volume of distribution 9.9 l/kg. The C_{max} after oral administration was observed at 30 min, which was the earliest time-point for blood sampling. The $AUC_{plasma,0–8\ h}$ was 5960 ± 240 ng·h/ml (mean ± SE) after i.v. administration and 1700 ± 130 and 3780 ± 270 ng·h/ml after oral administration of 25 and 80 mg/kg, respectively. The estimated oral bioavailability of zosuquidar·3HCl in mice was about 23% at 25 mg/kg and 16% at 80 mg/kg. The latter value, however, may be an underestimation of the bioavailability since the $AUC_{8–\infty}$ was not used in the calculation but may contribute substantially to the overall $AUC_{0–\infty}$.

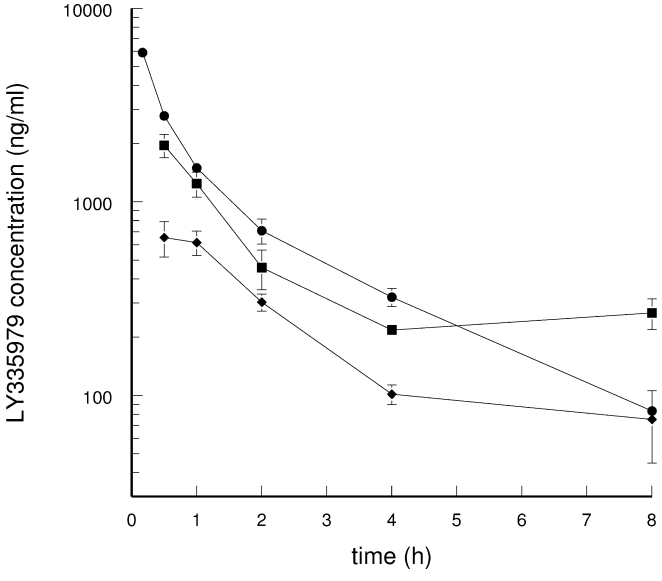

Fig. 2 Plasma concentration-time curves of zosuquidar·3HCl in wild-type mice after oral administration of 25 mg/kg (♦) and 80 mg/kg (●) zosuquidar·3HCl and after i.v. administration of 20 mg/kg (■) zosuquidar·3HCl

Discussion

This study showed that the P-gp inhibitor zosuquidar·3HCl was able to increase the penetration of paclitaxel into brain tissue. However, the inhibition of P-gp in the blood-brain barrier was only partially achieved, given the results in our reference P-gp knockout mouse model. Administration of zosuquidar·3HCl at 80 mg/kg orally or 20 mg/kg i.v. increased the brain concentration of paclitaxel fivefold to 45% of the levels found in the P-gp knockout mice. Relative to other orally administered P-gp inhibitors that we have tested in a previous study [9], it appears that zosuquidar·3HCl is more effective than cyclosporin A (24% of P-gp knockout), but less effective than elacridar (62% of P-gp knockout) and valspodar (56% of P-gp knockout). Thus, zosuquidar·3HCl increased the brain penetration of paclitaxel at best to about 45% of that observed in P-gp knockout mice and this value is in line with the results reported for the brain penetration of nelfinavir when given with i.v. zosuquidar·3HCl [3].

The effect of the plasma levels of zosuquidar·3HCl on the brain penetration and retention of paclitaxel is complex. There appears to be no clear relationship between the plasma concentration of zosuquidar·3HCl at the time of paclitaxel dosing and the brain penetration of paclitaxel. This suggests that maximum P-gp inhibition occurs when the concentration of zosuquidar·3HCl (and/or active metabolites, see below) in plasma has reached a certain threshold level. The idea of a threshold concentration would be in line with the results of a recent clinical study by Callies et al. [2] who showed that a plasma C_{max} above 200 μg/l is required to obtain maximal P-gp inhibition. By contrast, this putative threshold level of zosuquidar·3HCl appears to be less important for the brain retention of paclitaxel, since brain levels were sustained for up to 24 or 48 h after drug administration. This may indicate that a moderate inhibition of P-gp still occurs when plasma levels of zosuquidar·3HCl have declined to very low levels. Such a lasting inhibition of P-gp has also been reported in an

in vitro study where zosuquidar·3HCl inhibited P-gp in multidrug-resistant tumor cells, even after it was removed from the culture medium for several hours [5].

Surprisingly, a much lower brain penetration of paclitaxel was observed when i.v. administration of zosuquidar·3HCl was delayed from 10 min to 1 h before paclitaxel. This would suggest that the concentration of zosuquidar·3HCl in plasma 1 h after administration had already declined to levels that were no longer sufficient for effective inhibition of P-gp at the level of the blood-brain barrier. However, the plasma concentration of zosuquidar·3HCl 1 h after i.v. administration was in the same range as observed 1 h after oral administration of 80 mg/kg, the regimen resulting in maximally achievable inhibition of P-gp with zosuquidar·3HCl. The mechanism behind this discrepancy is unclear. Although speculative, an explanation may be that active metabolites were formed after oral administration of zosuquidar·3HCl, but not or less after i.v. administration.

When judging the efficacy of a P-gp inhibitor in increasing the brain concentration of paclitaxel, it is also necessary to take into account the influence of the inhibitor on the plasma concentration of paclitaxel. Treatment with zosuquidar·3HCl increased the paclitaxel concentrations in plasma to levels in the same range as in P-gp knockout control mice. This reduction in the clearance of paclitaxel probably reflects the inhibition of P-gp in the intestine by zosuquidar·3HCl. In mice P-gp-mediated excretion of unchanged paclitaxel in the gut accounts for a substantial part of the overall fecal elimination of unchanged drug [18]. In humans, however, this effect of P-gp inhibitors on excretion of unchanged paclitaxel will be much less (1.3-fold increase of paclitaxel AUC in the case of zosuquidar·3HCl [1]), because more than 90% of the administered dose will leave the body as metabolic product [10, 20].

Besides inhibition of P-gp, the reduction in paclitaxel clearance may also be a result of inhibition of other enzymes or transporter proteins involved in the elimination of paclitaxel. For example, coadministration of the P-gp inhibitors cyclosporin A and valspodar resulted in paclitaxel levels in plasma and tissues that were considerably higher than in P-gp knockout mice [9]. Most likely, cyclosporin A and PSC833 reduce the clearance of paclitaxel by inhibition of the murine equivalent of the human cytochrome P450 isoenzyme CYP3A. Our results are in line with the assumption that zosuquidar·3HCl is a much more specific P-gp inhibitor. Although there is evidence that the isoenzyme CYP3A4 is involved in the metabolism of zosuquidar·3HCl [7], it has been shown that zosuquidar·3HCl does not affect P450 isoenzymes at concentrations below the micromolar range [5]. Moreover, previous studies have also shown that zosuquidar·3HCl is a selective inhibitor of P-gp because it does not inhibit the drug transporter proteins MRP-1, MRP-2 or BCRP [5].

In patients, zosuquidar·3HCl appears to be well tolerated. However, central nervous system toxicity, characterized by cerebellar dysfunction, hallucinations and palinopsia, are dose-limiting for this drug [12]. With a current oral schedule of 500 mg/m^2 12-hourly for two doses, the C_{max} of zosuquidar·3HCl in patients is 450 μg/l. Schedules of 550 mg i.v. over 6 h and 400 mg i.v. over 3 h both result in a C_{max} of zosuquidar·3HCl in patients between 600 and 700 μg/l. These levels are lower than the plasma levels of zosuquidar·3HCl observed in mice (Fig. 2). Moreover, these higher plasma levels in mice were still insufficient to inhibit P-gp at the level of the blood-brain barrier completely. We therefore expect that the maximal achievable concentration of zosuquidar·3HCl in plasma of patients is also not sufficient to inhibit P-gp in the blood-brain barrier and to increase the brain penetration of paclitaxel. Moreover, patients with brain tumors often suffer from neurological dysfunction, making drugs with neurological side effects contraindicated.

In summary, zosuquidar·3HCl is a potent inhibitor of P-gp with moderate activity at the blood-brain barrier. The ability of P-gp in the blood-brain barrier to extrude paclitaxel from the brain appears to be readily restored when the zosuquidar·3HCl plasma levels decline. In patients, dose-limiting neurological toxicity by zosuquidar·3HCl occur at plasma levels of zosuquidar·3HCl which appear to be insufficient to inhibit P-gp at the blood-brain barrier [12]. This complication renders zosuquidar·3HCl unsuitable for clinical studies testing the concept of improving the penetration of paclitaxel into brain tumors by inhibition of P-gp.

Acknowledgements The authors thank A.J. Schrauwers for excellent biotechnical assistance. This work was supported by grant NKB992033 from the Dutch Cancer Society.

References

1. Callies S, de Alwis DP, Harris A, Vasey P, Beijnen JH, Schellens JH, Burgess M, Aarons L (2003) A population pharmacokinetic model for paclitaxel in the presence of a novel P-gp modulator, zosuquidar trihydrochloride (LY335979). Br J Clin Pharmacol 56:46–56
2. Callies S, de Alwis DP, Wright JG, Sandler A, Burgess M, Aarons L (2003) A population pharmacokinetic model for doxorubicin and doxorubicinol in the presence of a novel MDR modulator, zosuquidar trihydrochloride (LY335979). Cancer Chemother Pharmacol 51:107–118
3. Choo EF, Leake B, Wandel C, Imamura H, Wood AJ, Wilkinson GR, Kim RB (2000) Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. Drug Metab Dispos 28:655–660
4. Dantzig AH, Shepard RL, Cao J, Law KL, Ehlhardt WJ, Baughman TM, Bumol TF, Starling JJ (1996) Reversal of P-glycoprotein-mediated multidrug resistance by a potent cyclopropyldibenzosuberane modulator, LY335979. Cancer Res 56:4171–4179
5. Dantzig AH, Shepard RL, Law KL, Tabas L, Pratt S, Gillespie JS, Binkley SN, Kuhfeld MT, Starling JJ, Wrighton SA (1999) Selectivity of the multidrug resistance modulator, LY335979, for P-glycoprotein and effect on cytochrome P-450 activities. J Pharmacol Exp Ther 290:854–862
6. Dantzig AH, Law KL, Cao J, Starling JJ (2001) Reversal of multidrug resistance by the P-glycoprotein modulator, LY335979, from the bench to the clinic. Curr Med Chem 8:39–50

7. Ehlhardt WJ, Woodland JM, Baughman TM, van den Branden M, Wrighton SA, Kroin JS, Norman BH, Maple SR (1998) Liquid chromatography nuclear magnetic resonance spectroscopy and liquid chromatography mass spectrometry identification of novel metabolites of the multidrug resistance modulator LY335979 in rat bile and human liver microsomal incubations. Drug Metab Dispos 26:42–51
8. Kemper EM, Ouwehand M, Beijnen JH, van Tellingen O (2003) Bioanalysis of zosuquidar trihydrochloride (LY335979) in small volumes of human and murine plasma by ionpairing reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Appl (in press)
9. Kemper EM, van Zandbergen AE, Cleypool C, Mos HA, Boogerd W, Beijnen JH, van Tellingen O (2003) Increased penetration of paclitaxel into the brain by inhibition of P-glycoprotein. Clin Cancer Res 9:2849–2855
10. Malingre MM, Schellens JHM, van Tellingen O, Rosing H, Koopman FJ, Duchin K, ten Bokkel Huinink WW, Swart M, Beijnen JH (2000) Metabolism and excretion of paclitaxel after oral administration in combination with cyclosporin A and after i.v. administration. Anticancer Drugs 11:813–820
11. Proost JH, Meijer DK (1992) MW/Pharm, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. Comput Biol Med 22:155–163
12. Rubin EH, de Alwis DP, Pouliquen I, Green L, Marder P, Lin Y, Musanti R, Grospe SL, Smith SL, Toppmeyer DL, Much J, Kane M, Chaudhary A, Jordan C, Burgess M, Slapak CA (2002) A phase I trial of a potent P-glycoprotein inhibitor, zosuquidar.3HCl trihydrochloride (LY335979), administered orally in combination with doxorubicin in patients with advanced malignancies. Clin Cancer Res 8:3710–3717
13. Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CA, van der Valk MA, Robanus-Maandag EC, te Riele HP (1994) Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell 77:491–502
14. Schinkel AH, Wagenaar E, Mol CAAM, Vandeemter L (1996) P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 97:2517–2524
15. Shepard RL, Cao J, Starling JJ, Dantzig AH (2003) Modulation of P-glycoprotein but not MRP1- or BCRP-mediated drug resistance by LY335979. Int J Cancer 103:121–125
16. Slate DL, Bruno NA, Casey SM, Zutshi N, Garvin LJ, Wu H, Pfister JR (1995) RS-33295-198: a novel, potent modulator of P-glycoprotein-mediated multidrug resistance. Anticancer Res 15:811–814
17. Sparreboom A, van Tellingen O, Nooijen WJ, Beijnen JH (1995) Determination of paclitaxel and metabolites in mouse plasma, tissues, urine and faeces by semi-automated reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Appl 664:383–391
18. Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DKF, Borst P, Nooijen WJ, Beijnen JH, van Tellingen O (1997) Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci U S A 94:2031–2035
19. Starling JJ, Shepard RL, Cao J, Law KL, Norman BH, Kroin JS, Ehlhardt WJ, Baughman TM, Winter MA, Bell MG, Shih C, Gruber J, Elmquist WF, Dantzig AH (1997) Pharmacological characterization of LY335979: a potent cyclopropyldibenzosuberane modulator of P-glycoprotein. Adv Enzyme Regul 37:335–347
20. Walle T, Walle UK, Kumar GN, Bhalla KN (1995) Taxol metabolism and disposition in cancer patients. Drug Metab Dispos 23:506–512