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# Improved penetration of docetaxel into the brain by co-administration of inhibitors of P-glycoprotein

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**Abstract**

P-glycoprotein (Pgp) in the blood–brain barrier limits the brain's uptake of many anticancer drugs. We have investigated whether the Pgp inhibitors cyclosporin A, valspodar (PSC833) and elacridar (GF120918) increase the accumulation of docetaxel in the brain. Pgp knockout mice served as a reference model for the complete absence or complete inhibition of Pgp. Plasma and tissues were analysed by high-performance liquid chromatography. Cyclosporin A, valspodar and elacridar significantly increased the brain concentrations of docetaxel in wild-type mice to 38%, 56% and 59%, respectively, of those achieved in Pgp knockout mice. Valspodar and cyclosporin A also increased the docetaxel concentration in plasma and other tissues by 2- and 3-fold, whereas elacridar did not change the clearance. All three inhibitors therefore inhibit Pgp in the blood–brain barrier. Elacridar increases the accumulation of docetaxel in the brain without significant effects on systemic exposure. Further clinical tests with this latter combination are warranted.

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*Keywords:* Docetaxel; P-glycoprotein; Blood–brain barrier; Elacridar; Valspodar; Cyclosporin A

## 1. Introduction

Docetaxel (Taxotere®) is an effective drug against various malignancies, including ovarian and breast cancer [1]. However, the efficacy of systemic treatment with docetaxel against tumours or metastases in the brain is limited [2]. Probably a major cause of this lack of efficacy is the blood–brain barrier (BBB), which restricts the penetration of drugs into the brain tumour tissue. An important component of the BBB is P-glycoprotein (Pgp), which acts as a drug efflux pump. Studies in Pgp knockout mice, the reference model for the absence or 'complete inhibition' of Pgp, have shown that Pgp limits the entry of anticancer drugs such as vinblastine, doxorubicin and paclitaxel into the brain [3–5].

Inhibition of Pgp may, therefore, be an attractive strategy for increasing the penetration of these and other Pgp-substrate anticancer drugs into the brain. Fellner and colleagues showed improved efficacy of paclitaxel against experimental brain tumours when given in combination with the Pgp-inhibitor valspodar [6]. We have recently shown that potent Pgp inhibitors such as valspodar (PSC833) and elacridar (GF120918) can enhance the brain's uptake of paclitaxel substantially (5-fold), but not completely, as the concentrations achieved were still only 50% of those observed in Pgp knockout mice [5]. Apparently, paclitaxel is such a good substrate for Pgp that any remaining Pgp pump activity still results in a substantial efflux of paclitaxel. Studies *in vitro* indicate that docetaxel may be a weaker substrate

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for Pgp than paclitaxel [7,8]. We therefore hypothesised that it might be easier to inhibit the efflux of docetaxel by Pgp in the BBB and to enhance the uptake of docetaxel into the brain by the co-administration of an inhibitor of Pgp.

The aim of this study was to determine the importance of Pgp in the distribution of docetaxel into the brain and to establish to what extent the penetration of this drug into the brain can be increased by co-administration of the Pgp inhibitors valspodar, elacridar and cyclosporin A., We also included Pgp knockout mice in our studies as a reference for complete inhibition of Pgp in the BBB and to discover to what extent the increased plasma concentrations contribute to any higher concentrations in the brain.

## 2. Materials and methods

### 2.1. Laboratory animals

Experiments were carried out on female FVB wild-type and Pgp knockout (*mdr1ab(−/−)*) mice [9], 10–14 weeks of age. Food (Hope Farms B.V., Woerden, The Netherlands) and acidified water were provided *ad libitum*. Animals were handled in accordance with Dutch national law.

### 2.2. Preparation of drug solutions

Docetaxel (Taxotere®) was obtained from Aventis (Anthony Cedex, France). A solution of 10 mg/ml formulated in Tween 80:ethanol:saline (20:13:67, v/v/v) was diluted on the day of injection with sterile saline (Braun, Emmer-Compascuum, The Netherlands) to a final concentration of 3.3 mg/ml. A solution of 50 mg/ml cyclosporin A (Novartis Pharma, Basel, Switzerland) formulated in cremophor EL-ethanol (67:33, v/v) was diluted on the day of administration to 5 mg/ml with saline. Valspodar (PSC833) was kindly provided by Novartis Pharma (Basel, Switzerland). A 50 mg/ml stock solution of valspodar in ethanol:cremophor EL (1:1, v/v) was diluted with saline to a final concentration of 2.5 mg/ml. Elacridar·HCl (GF120918) was kindly provided by GlaxoSmithKline (Research Triangle Park, NC, USA). Elacridar suspensions of 2.5 mg/ml (free base) were prepared in 5 g/l of hydroxypropyl methyl cellulose (Sigma Chemicals Co., St. Louis, MO, USA) and 1% Tween 80 (Sigma) in water for injection [10].

### 2.3. Pharmacokinetic study design

Docetaxel (33 mg/kg) was administered by intravenous injection to wild-type and Pgp knockout mice. The control groups received docetaxel alone. The other treatment groups received a single dose of cyclosporin A (50 mg/kg), valspodar (25 mg/kg) or elacridar (25 mg/kg) orally by gavage into the stomach at 1 h (cyclosporin A and valspodar) or 2 h (elacridar) before the docetaxel. The timing of these administrations was based on previous pharmacokinetic studies showing that peak plasma concentrations of elacridar occur about 1 h later than for cyclosporin A or valspodar [10]. Blood and tissues were collected at 1, 4, 8 and 24 h after docetaxel administration. In the wild-type and Pgp-knockout control groups, sampling was also done at 48 h after docetaxel administration. Four to six mice were used at each time point.

Blood was obtained by cardiac puncture under anaesthesia with methoxyflurane (Medical Developments Australia, Melbourne, Australia), transferred in tubes containing potassium EDTA and plasma was obtained by centrifugation (10 min, 3000 *g*). Next, the animals were killed by cervical dislocation and tissues (brain, liver, kidneys, lungs and heart) were removed and homogenised in 4% bovine serum albumin (Roche Diagnostics, Mannheim, Germany) in water (0.1–0.2 g/ml). Tissue homogenates and plasma were stored at −20 °C until analysis.

### 2.4. Analytical assay of docetaxel

Docetaxel was determined by a validated reversed-phase high-performance liquid method with ultraviolet detection, as described previously [11]. Docetaxel was extracted from plasma and tissue homogenates by a double liquid-liquid extraction with diethyl ether followed by a solid-phase extraction. Using 200 μl sample, the lower and upper limits of quantitation are 50 and 5000 ng/ml, respectively.

### 2.5. Pharmacokinetic and statistical analysis

The area under the concentration–time curve (AUC) and the standard error of the AUC ($SE_{AUC}$) of docetaxel in plasma and tissues were calculated by the linear trapezoidal rule from time point 0 to the last sample with a concentration above the lower limit of quantitation. For formulae, see Ref. [10]. The standard error of the ratio of the $AUC_{tissue}$ and $AUC_{plasma}$ was calculated by the formula:

$$SE_{ratio} = ratio \times \sqrt{\left(\frac{SE_{AUC_{tissue}}}{AUC_{tissue}}\right)^2 + \left(\frac{SE_{AUC_{plasma}}}{AUC_{plasma}}\right)^2}$$

The elimination half-life of docetaxel was calculated using the software program *MW/Pharm* (Mediware, Groningen, The Netherlands) [12]. The two-sided unpaired Student *t*-test was used for statistical analysis. Because of the multiple-comparison analyses a *P*-value of 0.01 or less was regarded as statistically significant.

## 3. Results

The administration of docetaxel to wild-type mice resulted in low concentrations in the brain (Fig. 1). In our Pgp knockout mice the concentration of docetaxel in the brain was between 4- and 9-fold higher at the various time points. The exposure of the brain to docetaxel was calculated from the $AUC_{brain,0-24}$ (Table 1) and increased by 6.2-fold from 2.5±0.2 μg/h per g (mean±SE) in the wild-type mice to 15.4±0.5 μg/h per g in the Pgp knockout mice ($P<0.001$). The very slow elimination of docetaxel from the brain of both wild-type and Pgp knockout mice, resulting in measurable amounts even at 48 h after administration, is noteworthy.

Administration of cyclosporin A, valspodar and elacridar to the wild-type mice resulted in a significantly increased concentration of docetaxel in the brain (Fig. 2). However, none of the inhibitors at the investigated doses was able to increase the concentrations of docetaxel in the brains of wild-type mice to those achieved in the Pgp knockout mice. Overall, the $AUC_{brain,0-24}$ increased by 2.3-fold for cyclosporin A, 3.5-fold for valspodar and 3.6-fold for elacridar (Table 1), corresponding to 38% for cyclosporin A, 56% for valspodar and 59% for elacridar relative to the concentrations found in the Pgp knockout controls.

In this study, we also included Pgp knockout mice receiving a combination of docetaxel and cyclosporin A, valspodar or elacridar. The brain concentration in the Pgp knockout control mice and the Pgp knockout mice receiving cyclosporin A and valspodar was significantly higher, whereas elacridar left the brain concentration unchanged. The greatest enhancement was observed with valspodar; based on the brain-to-plasma ratio this was mainly driven by the higher plasma concentration (Table 1). In marked contrast to this finding, the even higher plasma concentrations of docetaxel when cyclosporin A was given did not result in a substantially higher accumulation of docetaxel in the brain, and thus to a reduction in the brain-to-plasma ratio. Moreover, only in the case of cyclosporin A was there a significant decline in the brain concentration of docetaxel between 1 and 4 h after drug administration. Taking into account that co-administration of cyclosporin A results in substantially higher plasma concentrations at 1 h after drug administration, it seems plausible that the high concentrations of docetaxel in the brain at 1 h actually represent drug present in the blood compartment.

In contrast with the concentrations of docetaxel in the brain, those in plasma in both wild-type and Pgp knockout mice declined more rapidly, with an elimination half-life of about 1 h (Fig. 3). The plasma concentration time profiles of docetaxel in wild-type and Pgp knockout mice were not significantly different (Fig. 3) and did not change after co-administration of elacridar (Fig. 3; Table 1). The plasma concentration of docetaxel in wild-type mice receiving cyclosporin A or valspodar, however, was significantly increased, by 3.1- and 1.8-fold, respectively ($P<0.001$) (Table 1). In the case of valspodar, the $AUC_{plasma,0-8}$ in the Pgp knockout mice was further increased relative to the wild-type mice.

The ratios of the AUC of the tissues and plasma (tissue/plasma ratio) were used to determine the influence of the plasma concentration on the concentrations in tissues. The brain-to-plasma ratio was less than 1 for the wild-type control mice and wild-type mice receiving an inhibitor of Pgp. No difference was observed between the ratios for the wild-type control group and the wild-type mice receiving cyclosporin A, whereas the administration of valspodar and elacridar to wild-type mice

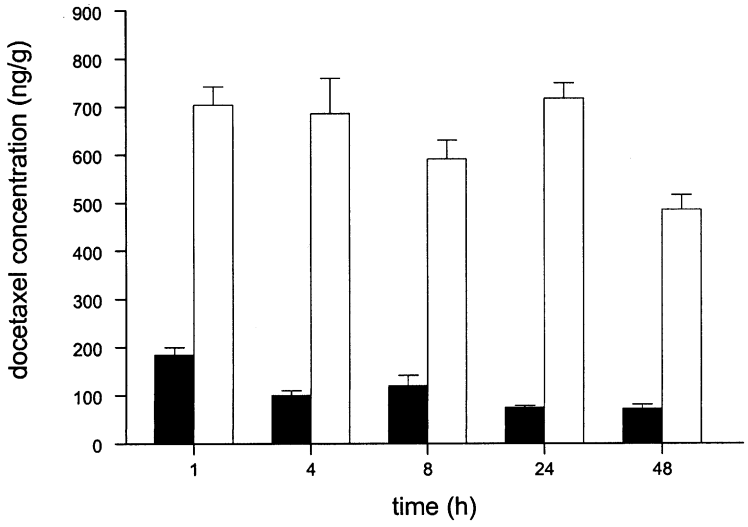


Fig. 1. Brain concentrations of docetaxel in wild-type (closed bars) and Pgp knockout mice (open bars) at 1, 4, 8, 24 and 48 h after administration of 33 mg/kg docetaxel. Each bar represents the mean±SEM for 5–6 mice.

Table 1
Area under the concentration time curves (AUC) (mean±SE) of docetaxel in plasma (μg/h per ml) and tissues (μg/h per g)

| | | Plasma (0–8 h) | Brain (0–24 h) | Brain-to-plasma ratio | Liver (0–24 h) | Liver-to plasma ratio | Kidneys (0–24 h) | Kidney-to plasma ratio | Lungs (0–24 h) | Lungs-to-plasma ratio | Heart (0–8 h) | Heart-to-plasma ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Wild type | Control | 8.7±0.9 | 2.5±0.2 | 0.29±0.04 | 40.6±5.4 | 4.7±0.8 | 57.5±3.0 | 6.6±0.8 | 61.4±2.4 | 7.1±0.8 | 59.2±4.5 | 6.8±0.9 |
| | Cyclosporin A | 27.0±2.2* | 5.8±0.5* | 0.21±0.02$^{ns}$ | 100±7* | 3.7±0.4$^{ns}$ | 139±12* | 5.1±0.6$^{ns}$ | 177±10* | 6.5±0.6$^{ns}$ | 158±14* | 5.9±0.7$^{ns}$ |
| | Valspodar | 15.9±1.8* | 8.7±0.7* | 0.55±0.08** | 55.5±6.0$^{ns}$ | 3.5±0.6$^{ns}$ | 101±5* | 6.4±0.7$^{ns}$ | 116±5* | 7.3±0.9$^{ns}$ | 83.8±3.1* | 5.3±0.6$^{ns}$ |
| | Elacridar | 12.4±1.4$^{ns}$ | 9.1±1.0* | 0.73±0.11* | 60.6±6.4$^{ns}$ | 4.9±0.7$^{ns}$ | 71.5±3.1** | 5.8±0.7$^{ns}$ | 97.8±5.0* | 7.9±1.0$^{ns}$ | 66.3±7.5$^{ns}$ | 5.3±0.8$^{ns}$ |
| Pgp knockout | Control | 9.0±1.4$^{ns}$ | 15.4±0.5* | 1.7±0.3* | 51.9±6.1$^{ns}$ | 5.8±1.1$^{ns}$ | 61.4±2.1$^{ns}$ | 6.8±1.1$^{ns}$ | 105±3* | 11.7±1.8$^{ns}$ | 66.5±2.6$^{ns}$ | 7.4±1.2$^{ns}$ |
| | Cyclosporin A | 27.4±1.8[a] | 17.9±0.7[b] | 0.65±0.05[a] | 86.3±5.0[†] | 3.1±0.3$^{NS}$ | 157±8[a] | 5.7±0.5$^{NS}$ | 219±13[a] | 8.0±0.7$^{NS}$ | 132±10[a] | 4.8±0.5$^{NS}$ |
| | Valspodar | 21.3±3.5[a] | 22.6±0.9[a] | 1.06±0.18$^{NS}$ | 63.8±5.8$^{NS}$ | 3.0±0.6$^{NS}$ | 126±10[a] | 5.9±1.1$^{NS}$ | 150±16 $^{NS}$ | 7.0±1.4$^{NS}$ | 93.5±9.0[a] | 4.4±0.8$^{NS}$ |
| | Elacridar | 11.5±3.0$^{NS}$ | 14.5±0.6$^{NS}$ | 1.26±0.33$^{NS}$ | 46.2±11.6$^{NS}$ | 4.0±1.4$^{NS}$ | 67.4±8.4$^{NS}$ | 5.9±1.7$^{NS}$ | 98.0±11.2$^{NS}$ | 8.5±2.4$^{NS}$ | 73.3±12.7$^{NS}$ | 6.4±2.0$^{NS}$ |

NS, Not significantly different compared to Pgp knockout control mice. ns, Not significantly different compared to wild-type control mice. *$P<0.001$ compared to wild-type control mice.
**$0.01<P<0.001$ compared to wild-type control mice.
[a] $P<0.001$ compared to Pgp knockout control mice.
[b] $0.01<P<0.001$ compared to Pgp knockout control mice.

increased the ratios by 1.9- and 2.5-fold, respectively ($P<0.01$).

The concentrations of docetaxel in the other tissues followed a similar pattern to that in plasma, which also is reflected in the tissue-to-plasma ratios (Table 1). In the Pgp knockout control mice only the concentrations in the lungs significantly differed from those observed in wild-type control mice ($P<0.01$) (Table 1). In wild-type mice receiving elacridar the AUC of docetaxel in the liver, kidney, lungs and heart were not significantly different from those of the Pgp knockout control mice. Treatment with cyclosporin A or valspodar resulted in a further increase of the AUC in these organs, but they were in the same range as those in the Pgp knockout mice receiving cyclosporin A or valspodar.

## 4. Discussion

This study shows that Pgp limits the penetration of docetaxel into the brain and that inhibitors of Pgp can help to increase this penetration significantly. The absence of Pgp in Pgp knockout mice did not reduce the plasma clearance of docetaxel. Similarly, the administration of a selective inhibitor of Pgp, such as elacridar, did not increase the concentrations of docetaxel in plasma and tissues other than the brain. Therefore, this inhibitor is the most promising candidate for further clinical trials to increase the brain penetration of docetaxel in patients with a brain tumour.

The Pgp inhibitors valspodar and elacridar were about equally effective in increasing the concentration of docetaxel in the brain of wild-type mice and more effective than cyclosporin A. Treatment with valspodar and elacridar did not result in complete inhibition of Pgp in the BBB. Using the Pgp knockout mice as a model of the complete inhibition of Pgp, we found that the brain concentrations after treatment with valspodar and elacridar were 53% and 56% of those in the Pgp knockout mice control mice. Although higher doses of Pgp inhibitors might result in more effective Pgp inhibition and thus better penetration and retention of docetaxel in the brain, a dose of as little as 25 mg/kg valspodar results in plasma concentrations of valspodar [5] that are known to cause serious side-effects (e.g. ataxia) in patients [13]. Consequently, a further increase in the dosage of valspodar does not appear clinically relevant. Further dose escalation with elacridar may be feasible. In patients, the maximum plasma concentration of elacridar was about 800 ng/ml after a single oral dose of 1000 mg without significant side-effect [14]. The plasma concentration achieved in mice receiving a dose of 25 mg/kg elacridar was about 500 ng/ml [10].

Administration of the Pgp inhibitors to Pgp knockout mice was very helpful in addressing the question of whether the increased plasma concentrations contribute

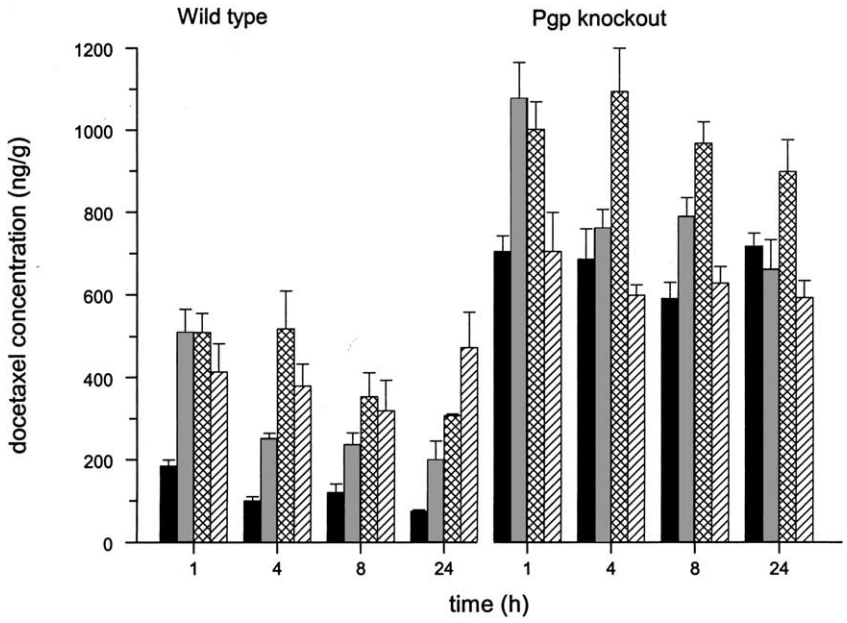


Fig. 2. Brain concentrations of docetaxel in wild-type and Pgp knockout mice at 1, 4, 8 and 24 h after administration of 33 mg/kg docetaxel alone (■) or combined with 50 mg/kg cyclosporin A (▩), 25 mg/kg valspodar (▨) and 25 mg/kg elacridar (▨). Each bar represents the mean ± SEM for 4–6 mice.

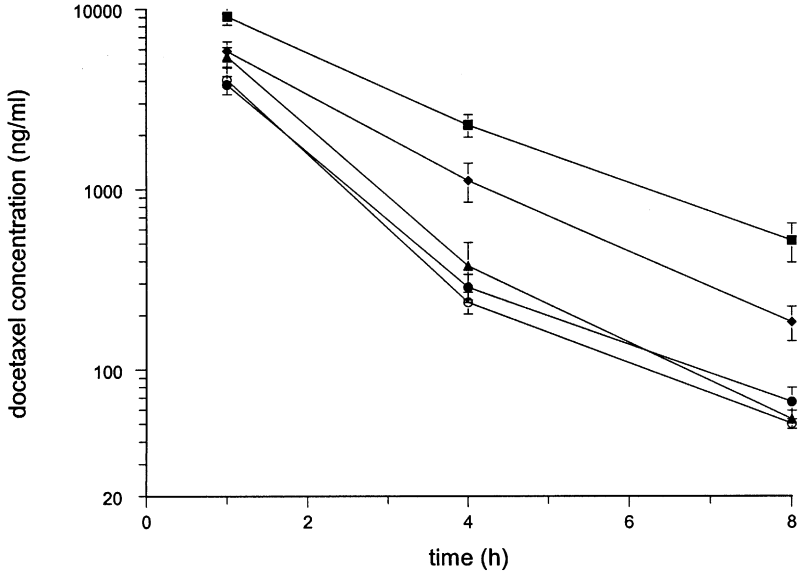


Fig. 3. Plasma concentration-time curves of docetaxel in wild-type mice after administration of 33 mg/kg docetaxel alone (●) and in combination with cyclosporin A (■), valspodar (◆), elacridar (▲) and docetaxel alone in Pgp knockout mice (○).

to the increase in the concentration of docetaxel in the brain. Cyclosporin A and valspodar increased the penetration of docetaxel into the brain of the Pgp knockout mice as compared to the Pgp knockout control group. Consequently, it is very likely that in wild-type mice also the increased brain concentration of docetaxel by cyclosporin A and valspodar is mediated at least in part by the higher plasma concentration. Because elacridar did not increase the plasma concentrations, it is likely that elacridar increased the brain penetration by inhibition of Pgp only.

The elimination of docetaxel from plasma is rapid. Interestingly, the clearance of docetaxel was not altered in Pgp knockout mice compared with wild-type mice. In many cases, the administration of a Pgp substrate to Pgp knockout mice results in diminished excretion of the substrate from the body [3–5]. Also, the administration of Pgp inhibitors frequently results in an increase in the concentration of the Pgp substrate in plasma [5,15,16], but here this occurred only with cyclosporin A and valspodar. Treatment with elacridar resulted in similar concentrations of docetaxel in plasma and tissues

to those in the wild-type control mice. This increased systemic exposure to docetaxel with cyclosporin A and valspodar will probably lead to an enhancement of the toxicity of this drug (e.g. bone marrow suppression and neurotoxicity).

The increased concentrations of docetaxel in Pgp knockout mice receiving cyclosporin A or valspodar compared to those in Pgp knockout controls show that the interaction caused by these compounds and docetaxel is not only due to inhibition of Pgp, but also to other factors (e.g. inhibition of cytochrome P450 isoenzymes and possibly also other transport proteins).

It appears that Pgp is less efficient in protecting the brain against docetaxel than is paclitaxel. With paclitaxel we recently found a 11-fold difference in brain penetration between Pgp knockout and wild-type mice [5], whereas this was only 6.2-fold with docetaxel. This result is in line with the finding that docetaxel appears to be a weaker substrate for Pgp than paclitaxel [8]. Consequently, we expected that a weaker Pgp inhibitor, such as cyclosporin A, might be more efficacious than paclitaxel in increasing the brain penetration of docetaxel. For example, a study by Hendrikse and colleagues [17] showed that cyclosporin A increased the brain penetration of verapamil to the same as in Pgp knockout mice. However, the higher brain concentrations of docetaxel by cyclosporin A found here were primarily due to the substantially higher plasma concentrations.

In conclusion, docetaxel appears to be an interesting drug for further clinical studies in brain tumour patients. It should be given in combination with a potent Pgp inhibitor. Because the clearance of docetaxel is not dependent on the activity of Pgp, a selective Pgp inhibitor, such as elacridar, may allow the administration of docetaxel at full doses.

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