Screening of Multidrug-Resistance Sensitive Drugs by *In Situ* Brain Perfusion in P-Glycoprotein-Deficient Mice

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Purpose. This study was conducted to assess the influence of P-glycoprotein (P-gp) on brain uptake of multidrug resistance sensitive drugs using an *in situ* brain perfusion technique in P-gp-deficient (mdr1a[–/–]) and wild-type mice.

Methods. The blood–brain transport of radiolabeled vinblastine, vincristine, doxorubicin, colchicine, and morphine was evaluated in mdr1a(–/–) and wild-type CF-1 mice with the *in situ* brain perfusion technique. Brain uptake of drugs after intravenous pretreatment with P-gp reversal agents, (PSC 833, GF 120918, or (±)-verapamil), or vehicle also was studied in wild-type mice. In all experiments, cerebral vascular volume was determined by co-perfusion of sucrose.

Results. Cerebral vascular volume was preserved during perfusion, indicating maintenance of blood–brain barrier integrity in both types of mice within the concentration range of substrates in the perfusate. The apparent brain transport of colchicine, vinblastine, doxorubicin, and morphine was increased 3.0, 2.7, 1.5, and 1.4-fold, respectively, in mdr1a(–/–) mice compared with the wild-type; the brain uptake of vincristine was not affected by P-gp. Preadministration of PSC 833 or GF 120918 in wild-type mice led to a ~3-fold increase in the brain transport of colchicine and vinblastine, but no effect was observed for the other compounds. Intravenous verapamil enhanced colchicine brain transport (1.8-fold), but failed to increase the brain uptake of vinblastine and morphine.

Conclusion. The *in situ* brain perfusion technique appears to be a sensitive and powerful tool for medium throughput screening of the brain uptake of multidrug resistance sensitive drugs. The effect of P-gp is characterized more efficiently with mdr1a(–/–) mice than by using modulators of P-gp in wild-type mice.

KEY WORDS: P-glycoprotein; mdr1a(–/–) mice; reversal agents; blood–brain barrier; *in situ* brain perfusion.

INTRODUCTION

Transport of drugs across the BBB is an important factor in the development of centrally active drug candidates. The BBB is defined mainly by the permeability properties of the cerebral microvessels in which endothelial cells are linked by tight junctions that restrict the transfer of compounds from the systemic circulation to the brain (1). Recently, the multidrug resistance pump P-gp was identified as an important determinant of drug permeability across the BBB (2). P-gp is localized at the luminal membrane of the endothelial cells and extrudes a wide spectrum of substrates generally reported as hydrophobic, amphipathic, or cationic from the cells to the blood vessel lumen (3). In rodents, P-gp expression at the BBB is controlled by the mdr1a gene (4). Genetic engineering and a spontaneous mutation in the mdr1a gene have led to the development of P-gp-deficient mouse strains referred to as mdr1a(–/–) (5,6). *In vitro* models that reconstitute a monolayer of cerebral capillary endothelial cells have been proposed as a rapid or high throughput screening procedure to assess BBB permeability of drug candidates (7). More recently, because of the lack of agreement concerning *in vitro* BBB models, the Caco-2 cell model used to study intestinal drug absorption has been proposed for the prediction of drug penetration through the BBB (8). This lack of an accepted *in vitro* model to predict drug transport to the brain prompted us to search for alternative models. We recently described the *in situ* mouse brain perfusion model, which offers considerable advantages resulting from the availability of genetically engineered mice expressing defects in receptors, metabolic enzymes, and drug transporters such as the mdr1a(–/–) strain (9). The brain perfusion technique developed earlier in the rat by Takasato, et al. (10) represents a highly sensitive means to study BBB transport of drug candidates in terms of rate and mechanisms of transport (diffusion, facilitated, or active transport, efflux process) over a short period of time without the confounding factor of systemic disposition. In the present work, the *in situ* brain perfusion technique is applied to mice expressing or lacking mdr1a P-gp to assess the influence of several well-characterized multidrug resistance substrates and chemical modulators of P-gp. Finally, the method is discussed in terms of low, medium, or high throughput screening performance.

MATERIALS AND METHODS

Drugs and Chemicals

[^{3}H]-Vinblastine (12.5 Ci/mmol), [^{3}H]-vincristine (6.6 Ci/mmol), [^{14}C]-doxorubicin (55 mCi/mmol), and [^{14}C]-sucrose (565 mCi/mmol) were purchased from Amersham Pharmacia Biotech (Orsay, France). [^{3}H]-Morphine (83.5 Ci/mmol), [^{3}H]-colchicine (61.4 Ci/mmol), and [^{3}H]-sucrose (10.2 Ci/mmol) were purchased from New England Nuclear Life Sciences (Brussels, Belgium). GF 120918 was a gift from Glaxo Wellcome (Les Ulis, France). PSC 833 (Valspodar) was a gift from Novartis (Basel, Switzerland). Heparin sodium was obtained from Sanofi & Synthelabo, (Gentilly, France). (±)-Verapamil hydrochloride was purchased from Sigma (St Quentin Fallavier, France). All other chemicals were commercial products of analytical grade.

Animals

Adult male CF-1 mice (mdr1a[+/+] and [–/–], 30–40 g, 6–8 weeks old) were bred in-house from progenitors geno-

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ABBREVIATIONS: BBB, blood-brain barrier; P-gp, P-glycoprotein; CNS, central nervous system; b.wt, body weight; K_{in}, transport coefficient; V_d, volume of distribution; mdr, multidrug resistance.

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typed for mdr1a P-gp that were initially obtained from Charles River Laboratories (Wilmington, MA, USA).

Animals were housed in a room with a controlled environment (22 ± 3°C; 55 ± 10% relative humidity) and maintained under a 12-hour dark:light cycle (light from 6:00 a.m. to 6:00 p.m.). Animals had access to food and tap water *ad libitum.* All animals procedures complied with the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

Mouse Brain Perfusion Technique

Surgical Procedure and Transport Studies

Mice were anesthetized by intraperitoneal injection of xylazine (Bayer, Puteaux, France; 8 mg/kg) and ketamine (Parke Davis, Courbevoie, France; 140 mg/kg). Blood–brain transport of the compounds was measured in mdr1a(–/–) and wild-type mice using the *in situ* brain perfusion technique recently described in this laboratory (9). Briefly, the right common carotid was catheterized with polyethylene tubing (0.30 mm i.d. × 0.70 mm o.d., Biotrol Diagnostic, Chennevières-les-Louvre, France) filled with heparin (25 U/ml) and mounted on a 26-G needle. Before insertion of the catheter, the common carotid artery was ligated caudally. The external carotid was ligated rostral to the occipital artery at the level of the bifurcation of the common carotid artery. During surgery, body temperature was maintained from 37°C to 38°C using a rectal thermistor connected to a temperature monitor. The syringe containing the perfusion fluid was placed in an infusion pump (Harvard pump PHD 2000, Harvard Apparatus, Holliston, MA, USA) and connected to the catheter. Before perfusion, the thorax of the animal was opened, the heart was cut, and perfusion was started immediately with a flow rate of 2.5 ml/min. The perfusion fluid consisted of bicarbonate-buffered physiological saline (mM): 128 NaCl, 24 $NaHCO_3$, 4.2 KCl, 2.4 NaH_2PO_4, 1.5 $CaCl_2$, 0.9 $MgCl_2$, and 9 D-glucose. The solution was gassed with 95% O_2 and 5% CO_2 for pH control (7.4) and warmed to 37°C in a water bath. Tracers were added to perfusate at a concentration of 0.2–0.3 μCi/ml. Perfusion was terminated by decapitation at selected times (20, 30, 45, 60, 90 or 120 seconds). The brain was removed from the skull and dissected on ice. The right cerebral hemisphere was placed in tared vials and weighed. Aliquots of the perfusion fluid also were collected and weighed to determine tracer concentrations in the perfusate. Samples were digested in 1 ml of Solvable (Packard, Rungis, France) at 50°C and mixed with 9 ml of Ultima gold XR (Packard). Dual label counting was performed simultaneously in a Packard Tri-Carb model 1900 TR (Packard).

Calculation of BBB Transport Parameters

Brain vascular volume (V_{vasc}; μl/g) was estimated from the tissue distribution of [^{14}C]- or [^{3}H]-sucrose, which is known to diffuse very slowly across the BBB, using the following equation:

$$V_{vasc} = X^*/C^*_{perf} \quad (1)$$

where X* (dpm/g) is the amount of sucrose measured in the right brain hemisphere and C^*_{perf} (dpm/μl) is the concentration of labeled sucrose in the perfusion fluid.

Transport across the BBB was expressed in terms of two parameters: the apparent volume of distribution (V_{brain}) and the transport coefficient (K_{in}).

The apparent volume of distribution was calculated from the amount of radioactivity in the right brain hemisphere using the following equation:

$$V_{brain} = X_{brain}/C_{perf} \quad (2)$$

where X_{brain} (dpm/g) is the calculated amount of [^{14}C] or [^{3}H] tracer in the right cerebral hemisphere and C_{perf} (dpm/μl) is the labeled tracer concentration in the perfusion fluid. Brain tissue radioactivity was corrected for vascular contamination with the following equation:

$$X_{brain} = X_{tot} - V_{vasc} \cdot C_{perf} \quad (3)$$

where X_{tot} (dpm/g) is the total quantity of tracer measured in the tissue sample (vascular + extravascular).

Brain uptake also was expressed as a blood-brain transfer coefficient K_{in} (μl/s/g) and was calculated from:

$$K_{in} = V_{brain}/T \quad (4)$$

where T is the perfusion time (seconds).

The perfusion time used in single time point uptake studies was long enough to ensure that at least 40% total radioactivity in the tissue resided outside of the vascular space ($X_{brain} \geq 0.4\ X_{tot}$), (10).

Effects of PSC 833, GF 120918, and (±)-Verapamil as Reversing Agents

The chemical modulators of P-gp, GF 120918 (10 mg/kg), PSC 833 (10 mg/kg), or (±)-verapamil (1 mg/kg) were administered to wild-type mice and mdr1a(–/–) mice by a bolus injection (2 μl/g b.wt.) in the right femoral vein 5 minutes before brain perfusion according to the protocol of Drion et al (11). Control groups received the vehicle under the same conditions to assess a possible effect on brain transport. PSC 833 and GF120918 were dissolved in a 1:4 mixture of ethanol 99%:polyethylene glycol 200, and (±)-verapamil hydrochloride was dissolved in 0.9% saline before use. These solutions were protected from light and used within one day of preparation.

Statistical Analysis

Data are presented as mean ± standard deviation (S.D.) for 4–6 animals unless specified otherwise. Student's unpaired *t*-test was used to identify significant differences between groups when appropriate. In all cases the tests were two-tailed and statistical significance was set at $P < 0.05$.

RESULTS

Assessment of BBB Integrity

In all experiments, the physical integrity of the BBB was assessed with [^{3}H]- or [^{14}C]-sucrose. Co-perfusion of this vascular space marker in the presence of the radioactive compounds (vinblastine, vincristine, doxorubicin, morphine, or colchicine) was performed in both types of mice to assess potential opening of the BBB by these drugs and possible changes in BBB integrity after the disruption of the mdr1a gene in the deficient strain. The vascular volume in deficient

mdr1a mice did not differ significantly from that obtained in wild-type mice (Fig. 1). These results show that the vascular volumes were maintained during the transport experiment using the *in situ* brain perfusion technique in both types of mice within a drug concentration range of 3 nM to 3 μM and after 90 or 120 seconds of brain perfusion.

Time Course of Brain Uptake

To select an appropriate perfusion time, i.e., one that permits a sufficient accumulation of drug in brain tissue, a time–course study of brain distribution was performed for each compound in wild-type mice. Given a vascular volume of 14–16 μl/g, a distribution volume of 9–11 μl/g is equivalent to 40% of radioactivity residing in the extravascular space. This requirement was met following 90 or 120 seconds of perfusion with the radiolabeled compounds (Fig. 2).

Blood-Brain Transport Coefficients in Wild-Type and Mutant Mice for the Five Multidrug-Resistance Drugs

The blood–brain transfer coefficients (K_{in}) of the tested compounds after brain perfusion in both types of mice are shown in Fig. 3. The K_{in} values in wild-type mice ranged from 0.13 (vincristine) to 0.28 μl/s/g (doxorubicin). The K_{in} values for colchicine, vinblastine, doxorubicin and morphine were increased 3.0, 2.7, 1.5, and 1.4-fold in mdr1a(–/–) mice, respectively, compared with wild-type mice. The brain uptake of vincristine was not affected by P-gp status after 120 seconds of perfusion.

Effect of PSC 833, GF 120918, and Verapamil as P-gp-Reversing Agents

The effect of the solvents used to dissolve the P-gp modulators on brain transport was assessed in initial experiments. Brain uptake of vinblastine, morphine, doxorubicin, and colchicine did not differ significantly compared to mice receiving the PSC 833 and GF 120918 vehicle (polyethylene glycol 200-ethanol) or verapamil vehicle (saline) injected in the femoral vein five minutes before brain perfusion (data not shown). PSC 833 and GF 120918 had no effect on the brain transport of vinblastine and colchicine in mdr1a(–/–) mice (Fig. 4). In wild-type mice, pre-treatment with PSC 833 (10 mg/kg) increased the brain uptake of vinblastine and colchicine about 3.0-fold, but had no effect on the transport of morphine and doxorubicin (Fig. 5.). GF 120918 (10 mg/kg) increased the brain uptake of vinblastine and colchicine about 3-fold but had no significant effect on morphine uptake (Fig. 5). Pre-treatment with verapamil (1 mg/kg), one of the most frequently used P-gp modulator *in vitro*, resulted in a 1.8-fold increase in the brain transport of colchicine compared to control mice, but failed to enhance vinblastine and morphine brain uptake (Fig. 5).

DISCUSSION

One major limitation to the penetration of many central nervous system (CNS) agents across the BBB is active efflux from the endothelial cell membrane to the blood by P-gp. It has been shown by conventional pharmacokinetic studies that the absence of mdr1a P-gp in mice leads to significantly higher brain-to-blood ratios for P-gp-sensitive compounds (5,12). Overall pharmacokinetic investigations, i.e., plasma and tissue analysis over time, are not suitable for rapid screening of a large number of CNS drug candidates. The *in situ* brain perfusion technique has been used in the rat to investigate amino acid, hormone and drug permeability properties at the BBB (13). Its recent application to mice represents a new screening model for the determination of drug permeability across the BBB. This technique could provide valuable information, such as the safety profile of a compound in terms of non-opening of the BBB, its penetration through the BBB,

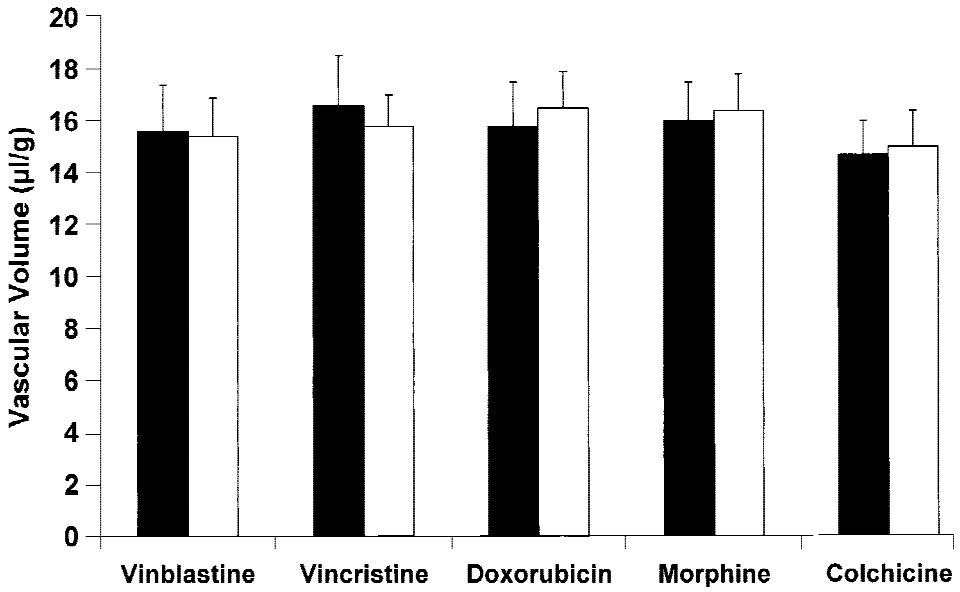

Fig. 1. Vascular volume (V_{vasc}, μl/g) in the right brain hemisphere of mdr1a(–/–) (open columns) and wild-type CF-1 mice (closed columns) determined from co-perfusion of radiolabeled sucrose with drugs tracers for 90 (colchicine) or 120 seconds (all other drugs). Each column represents the mean ± S.D. of 4–6 animals.

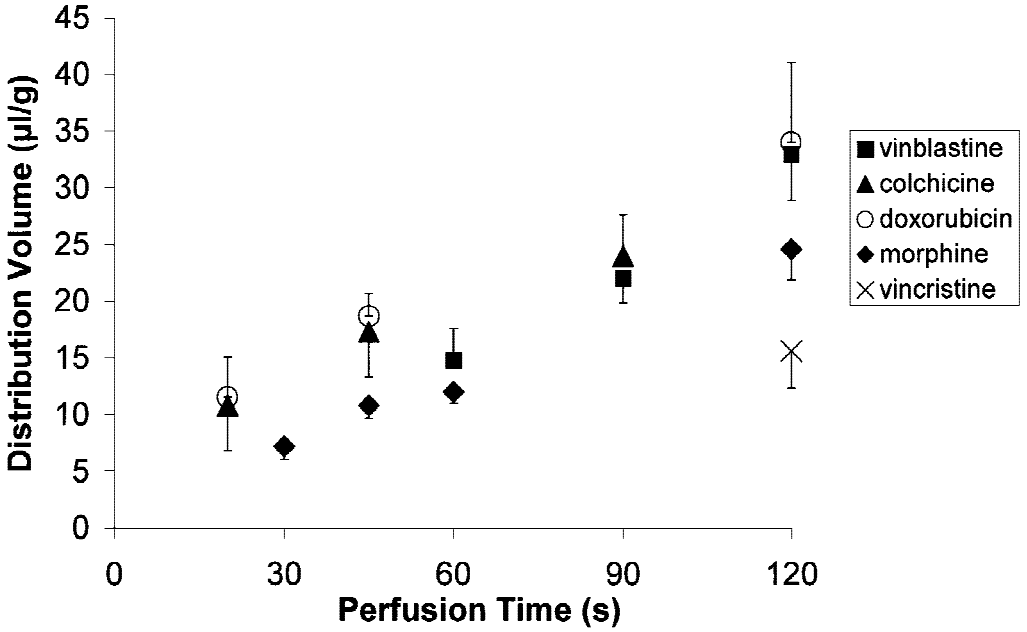

Fig. 2. Time–course of tracer (vinblastine, colchicine, doxorubicin, vincristine) uptake in the right brain hemisphere of wild-type mice, expressed as apparent brain distribution volume (V_{brain}, µl/g) determined using the *in situ* brain perfusion technique as described in methods. Data are presented as means ± S.D. (n = 4–9 animals per point).

as well as its interaction with P-gp. Application of *in situ* brain perfusion to wild-type and mdr1a(–/–) mice first required verification of BBB integrity. There was no difference in vascular volume determined from cerebral sucrose space between mdr1a(–/–) and wild-type mice during perfusion with studied compounds. The vascular volume agreed with previously reported values for co-perfusion of sucrose and inulin (9). These results indicate that the absence of P-gp and the presence of compounds in the perfusate (3 nM to 3 µM) were not associated with alteration of BBB integrity. In parallel studies, we noted that vinblastine concentrations have to be much higher (~200 µM) than the steady-state plasma level

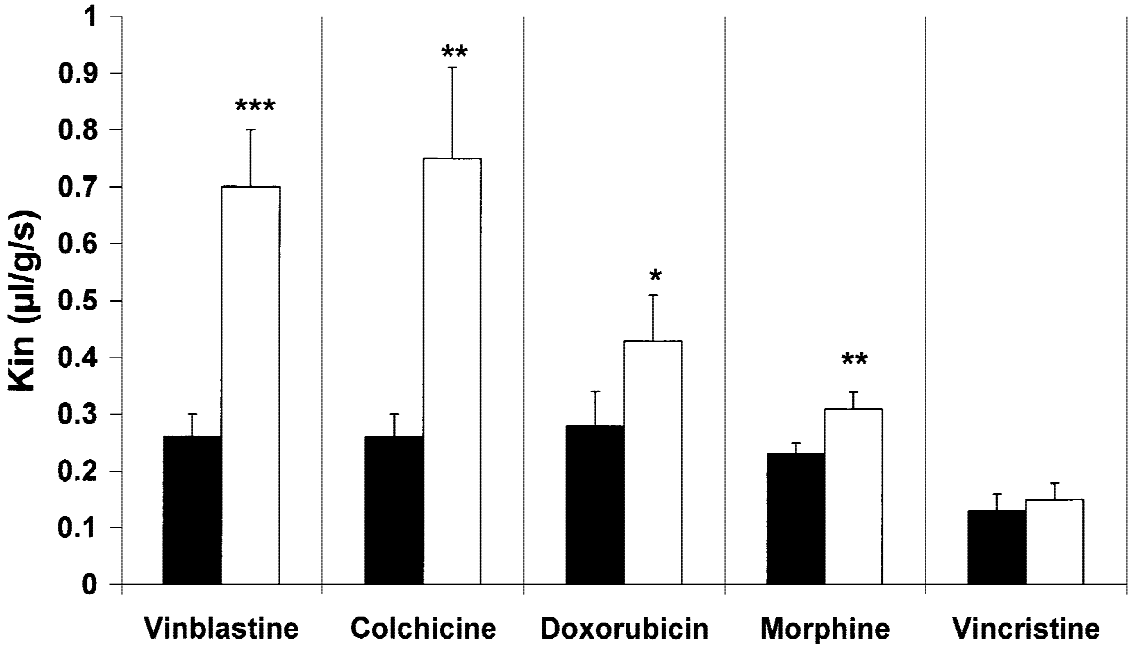

Fig. 3. Brain transport coefficient (K_{in}, µl/s/g) of tracers in wild-type (closed columns), and mdr1a(–/–) mice (open columns) measured with the *in situ* brain perfusion technique. Animals were perfused via the common carotid artery for 120 seconds with radiolabeled vinblastine, vincristine, doxorubicin and morphine, or 90 seconds with colchicine. Data are mean ± S.D. of 4–9 independent experiments. Statistically significant differences between wild-type and mdr1a(–/–) mice are indicated by * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

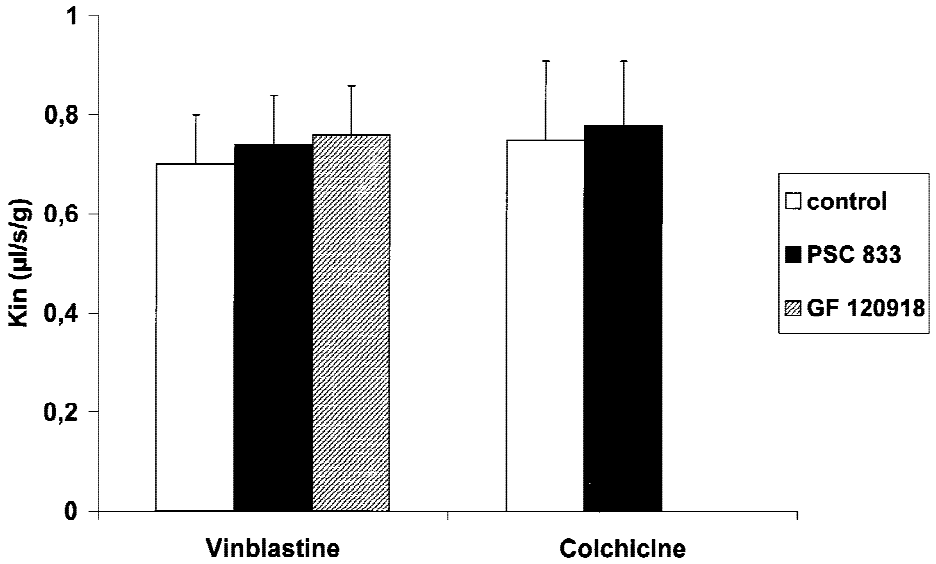

Fig. 4. Brain transport coefficient (K_{in}, µl/s/g) of vinblastine and colchicine measured with the *in situ* brain perfusion technique in the absence (control) or presence of reversal agents in P-gp deficient mdr1a(–/–) mice. P-gp modulators GF 120918 (10 mg/kg) or PSC 833 (10 mg/kg) were injected into the femoral vein 5 min before perfusion. Data are presented as means ± S.D. for $n = 3$–5 mice.

(~0.02 µM) (14) to significantly increase the sucrose space, which suggests opening of the BBB. Thus, this method could be used for the screening of compounds administered over a wide range of concentrations to investigate whether or not they open the BBB.

Another objective was to assess whether *in situ* brain perfusion in mice was suitable to study the penetration of compounds through the BBB and the potential influence of P-gp. We first noted that all tested drugs penetrate the BBB poorly in wild-type mice, as their K_{in} values ranged from 0.13 (vincristine) to 0.28 µl/s/g (doxorubicin). These values are of the same order of magnitude as that of the hydrophilic molecule urea (~0.1 µl/s/g) (9) and much lower than that of diazepam, a lipophilic compound that freely diffuses across the BBB and that is used to estimate flow (K_{in} = 42.5 µl/s/g) (9). The low brain penetration of the drugs used in our study in spite of their lipophilicity is probably due in part to their interaction with P-gp.

Screening of vinblastine, colchicine, morphine, doxorubicin, and vincristine with mdr1a(–/–) compared to wild-type

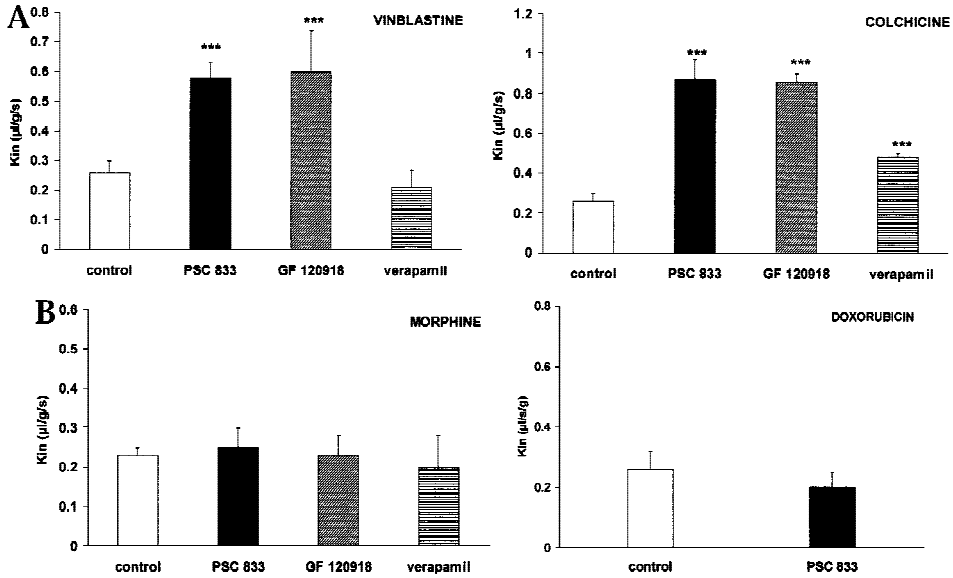

Fig. 5. Brain transport coefficient (K_{in}, µl/s/g) of vinblastine, colchicine (A), doxorubicin, and morphine (B) determined using the *in situ* brain perfusion technique in the absence (control) or presence of reversal agents in wild-type mice. P-gp modulators GF 120918 (10 mg/kg), PSC 833 (10 mg/kg), or (±)-verapamil (1 mg/kg) were injected into the femoral vein 5 minutes before perfusion. Data are presented as means ± S.D. for $n = 4$–6 mice. *** $P < 0.001$, comparing control group with treated group.

mice showed different profiles of results. First, the greatest difference between the two types of mice was found with vinblastine and colchicine, which both showed a ca. 3-fold increase in brain transport in mutant mice. Other studies have already indicated a strong influence of P-gp in limiting brain penetration of these two drugs (11,12). The brain-to-blood ratio of vinblastine has been reported to be higher in mdr1a(–/–) mice as compared to wild-type animals (15). Second, brain uptake of morphine and doxorubicin was enhanced about 1.5-fold in P-gp deficient compared to wild-type mice. This confirms, as shown in previous reports with transgenic and wild-type mice, that P-gp affects the transport of morphine across the BBB (12,16). After intravenous administration of morphine and doxorubicin in transgenic mice, the absence of P-gp moderately increased the brain-to-blood ratio of both morphine and doxorubicin as reported by other authors (12,16–18). This relatively weak impact of P-gp on the brain uptake of morphine and doxorubicin is consistent with the decreased ability of these two drugs to inhibit binding of the photoaffinity probe arylazidoprazosin to P-gp in rat brain capillaries as compared to vinblastine and colchicine (19). Finally, our results in wild-type mice confirm the lower brain penetration of vincristine (K_{in} = 0.13 μl/s/g) compared with vinblastine (K_{in} = 0.26 μl/s/g), two vinca alkaloids that differ in structure by a single chemical group, as already shown using *in situ* brain perfusion in the rat (20). Based on lipophilic prediction, the brain uptake observed is nonetheless 50 times lower than that predicted for vincristine from its logP value of 2.80 (21). The BBB remains an effective barrier to the transport of vincristine, even when the P-gp efflux pump is not expressed, as shown with mdr1a(–/–) mice after 2 min of brain perfusion. In one study, PSC 833 increased the accumulation of vincristine in P-gp-expressing tumors *in vivo* but had no influence on initial uptake in tumor cells *in vitro* (22). Interestingly, intravenous PSC 833 had no effect on the brain uptake of vincristine in rats *in vivo* (23,24). The results of *in vitro* studies in mdr cells suggest that P-gp handles the uptake and efflux of some substrates differentially (25). Taken together, these observations may suggest that the rate-limiting step for initial brain uptake of vincristine is upstream from the interaction with P-gp. Moreover, other efflux mechanisms at the level of the BBB, such as the possible expression of multidrug resistance proteins (MRP) known to interact with vincristine could be another mechanism responsible for this low penetration (26).

The role of P-gp can also be assessed by treating wild-type mice with P-gp reversing agents. Our results demonstrate that chemical inhibition of P-gp is not as effective as the mdr1a(–/–) mouse model to detect the influence of P-gp on the initial brain uptake of vinblastine, colchicine, morphine, and doxorubicin. We evaluated various P-gp inhibitors that belong to different pharmacological classes, including the calcium channel blocker verapamil, the cyclosporin analog PSC 833, and the acridonecarboxamide, derivative GF 120918. In our experiments with wild-type mice, pretreatment with PSC 833 or GF 120918 led to a 3-fold increase in vinblastine and colchicine brain transport, which is similar to that observed in mdr1a(–/–) mice. Furthermore, no enhancement effect of PSC 833 or GF 120918 on the brain uptake of vinblastine and colchicine was observed in mdr1a(–/–) mice. These data support that the increased in the brain uptake of vinblastine and colchicine in wild-type mice with the pretreatment of the two modulators was most likely because of P-gp. On the other hand, preadministration of verapamil in wild-type mice was only associated with a slight 1.8-fold increase in colchicine brain uptake and failed to increase vinblastine brain transport. This finding is in agreement with previous *in vivo* studies showing a lower inhibitory effect of verapamil than that observed with PSC 833 pretreatment in rat (11). The cardiovascular toxicity of verapamil precludes administration of doses that would result in systemic concentrations similar to those required to fully inhibit P-gp in most cell culture models (about 10 μM). In the present study, the dose-limiting serum concentration of 1–2 μM verapamil only partially inhibited P-gp mediated efflux (27). In contrast, the two other modulators PSC 833 and GF 120918 were administered at dose levels inhibiting P-gp (11,24,28). None of the P-gp inhibitors enhanced morphine or doxorubicin penetration into the brain. It is noteworthy that PSC 833 did not modify brain transport of doxorubicin. This has also been observed for other reversing agents such as cyclosporin A (29) and verapamil in the rat (30). The difference between genetic and chemical disruption of P-gp could be due to the inability of the pharmacological treatment to completely block P-gp. One of the reason could be the presence of multiple binding sites on P-gp (31,32). In such a case the modulators of P-gp may not bind the same site as the potential substrate thereby no effect of modulation could be seen. In addition, the lack of a significant increase in morphine and doxorubicin brain uptake by reversing agents could be the consequence of a limited contribution of P-gp on the apparent transport of these two compounds, consistent with the modest increases observed in P-gp deficient mice. Comparison of the mdr1a(–/–) mouse and reversing agents strategies clearly demonstrates that the first gives a more sensitive assessment of P-gp contribution in BBB transport than blocking P-gp functionality by pre-administration of P-gp modulators in wild-type mice.

From a scientific point of view, our data support the screening of CNS drug candidates potentially sensitive to P-gp using *in situ* brain perfusion in wild-type and mdr1a(–/–) mice. Several practical questions need to be addressed in order to evaluate the usefulness of this model in the drug development process. The surgical procedure took 15 minutes with a 95% success rate allowing study of two to three mice per hour per operator. Most failures could be attributed to rupture of the right carotid artery during catheterization. For our purposes, optimal brain perfusion time was 120 seconds. With this perfusion time, our technique required about 1.2 μCi of radiolabeled compound per mouse while a rat brain perfusion used 4.8 μCi. As radioactive compounds are not currently available in the early phase of drug development, cold compounds could also be perfused. This would require a sensitive and specific assay for the test compound in brain tissue. The current performance of liquid chromatography-mass spectrometry instruments should make it possible to extend the application of the *in situ* mouse brain perfusion technique to studies without radioactivity. Finally, for ethical and efficiency considerations, experimental groups of four animals might be considered acceptable to assess the influence of P-gp on brain uptake at a single time point. In this study, the inter-assay coefficient of variation ranged from 10–20%. Using Student's two-tailed *t* test with α = 5% and β = 20%, the smallest difference in brain uptake that can be detected between two groups of 4 wild-type and mdr1a(–/–)

mice at a single time point is a 1.3- and 1.8-fold increase with a coefficient of variation of 10 and 20%, respectively.

At present, there is no satisfactory established *in vitro* model of the BBB. However, such a model would be useful for high throughput screening during drug development. The *in situ* mouse brain perfusion technique could be used to rapidly assess the penetration of compounds through the BBB and their potential interaction with P-gp. Studies aimed at determining transport properties in terms of kinetic parameters (V_{max}, K_m) could also be performed with this method. Finally, the *in situ* mouse brain perfusion model could be used as a medium throughput screening method to evaluate the role of P-gp in the brain uptake of CNS drug candidates.

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