Pharmaceutical Research, Vol. 24, No. 9, September 2007 (© 2007)
DOI: 10.1007/s11095-007-9278-4

Short Communication

Modulation of the Brain Distribution of Imatinib and its Metabolites in Mice by Valspodar, Zosuquidar and Elacridar

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Received September 28, 2006; accepted February 20, 2007; published online March 23, 2007

Purpose. The selective protein tyrosine kinase inhibitor, imatinib, inhibits the growth of glioma cells in preclinical models, but its poor brain distribution limits its efficacy in patients. P-glycoprotein (P-gp, rodent Mdr1a/1b or Abcb1a/1b) and Breast cancer resistance protein (rodent Bcrp1 or Abcg2) were suggested to restrict the delivery of imatinib to the brain. This study evaluates the effect of administering selective inhibitors of these transporters together with imatinib on the systemic and cerebral disposition of imatinib in mice.

Materials and Methods. Wild-type, *Mdr1a/1b(−/−)* and *Bcrp1(−/−)* mice were given imatinib intravenously, either alone, or with valspodar, zosuquidar (P-gp inhibitors), or elacridar (a P-gp and Bcrp1 inhibitor). The blood and brain concentrations of [^{14}C]imatinib and its radioactive metabolites were determined.

Results. The blockade of P-gp by valspodar or zosuquidar (>3 mg/kg) enhanced the brain uptake of imatinib (~4-fold) in wild-type mice, but not that of its metabolites. Blockade of both P-gp and Bcrp1 by elacridar (>3 mg/kg) produced significantly greater brain penetration of imatinib (9.3-fold) and its metabolites (2.8-fold). In contrast, only the lack of P-gp enhanced imatinib brain penetration (6.4-fold) in knockout mice. These results of brain uptake correlated reasonably well with those obtained previously by our group using *in situ* brain perfusion.

Conclusions. Imatinib and its metabolites penetrate into the brain poorly and their penetration is limited by P-gp and (probably) Bcrp1. Administering imatinib together with P-gp (and Bcrp1) transporter inhibitors such as elacridar may improve the delivery of imatinib to the brain, making it potentially more effective against malignant gliomas.

KEY WORDS: brain; breast cancer resistance protein 1; imatinib; metabolites; P-glycoprotein.

INTRODUCTION

Imatinib (Gleevec®, formerly STI571) is a competitive inhibitor of ATP that blocks the tyrosine kinase activity of proteins such as ABL, BCR-ABL, c-KIT and platelet-derived growth factor receptors, whose deregulation is linked to the oncogenesis of certain tumors (1). Imatinib is indicated as a treatment for chronic myeloid leukaemia and gastrointestinal stromal tumors (2,3). Although imatinib was also active against malignant gliomas (MG) in preclinical experiments (4), it had little therapeutic benefit in clinical trials (5,6). This was probably due to its low ability to enter the central nervous system (CNS) of mammals (7–9). Imatinib had beneficial effects when it was administered to patients with MG in combination with other chemotherapy agents (10).

Insufficient drug delivery to the brain has been recognized as the a major cause of failure in CNS development programs (11,12). Besides having optimal pharmacokinetic properties (absorption, metabolism, elimination and plasma protein binding), CNS drug candidates must be able to cross the protective barriers of the brain. The blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier are the main functional interfaces that regulate the transfer of solutes between the systemic circulation and the brain (13). Both barriers express a wide variety of efflux transport proteins that limit the brain uptake of some lipophilic compounds. These include solute carrier transporters or ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp, human ABCB1, rodent Mdr1a/1b or Abcb1a/1b) and Breast cancer resistance protein (human BCRP or ABCG2, rodent Bcrp1 or Abcg2) (14–17).

Some recent preclinical studies have identified imatinib as a substrate and a modulator of both P-gp and BCRP/Bcrp1 (18–20). P-gp and Bcrp1 also limit the brain uptake of intravenous [^{14}C]imatinib in mice, suggesting that the blockade

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ABBREVIATIONS: ABC, ATP-binding cassette; BB ratio, Blood/brain concentration ratio; BBB, Blood–brain barrier; BCRP, Breast cancer resistance protein; CNS, Central nervous system; K_{net}, Net transport coefficient; MG, Malignant glioma; P-gp, P-glycoprotein; SD, Standard deviation.

of imatinib brain efflux by transporter modulators could enhance the delivery of the drug to the brain (7,21). Although Dai *et al.* showed that imatinib is extensively metabolized in mice (7), their drug–drug interaction study, and that of Breedveld *et al.* (21), were based on the distribution of total radioactivity in the brain, which did not decouple the effects of the transporter inhibitor on the brain penetration of unchanged imatinib and its radioactive metabolites. We previously investigated the mechanisms involved in the transport of unchanged imatinib at the mouse BBB by *in situ* brain perfusion.[2] Our results confirmed those of Dai *et al.* (7) showing the capacity of P-gp to restrict the brain penetration of imatinib. We also showed the probable additive or synergistic action of Bcrp1. However, although the *in situ* technique provides valuable information on the mechanism of drug transport across the BBB (22), it only explores the initial kinetics of brain uptake (23,24).

The "gold standard" for studying drug transport to the brain is the intravenous injection method, which allows the determination of the brain penetration at equilibrium and takes into account disposition processes like plasma protein binding, metabolism and elimination, and peripheral tissue distribution (25). The present study examined the concentration of imatinib and its metabolites in the blood and brain after the intravenous administration of imatinib to wild-type, *Mdr1a/1b(−/−)* and *Bcrp1(−/−)* knockout mice, alone or with the P-gp inhibitors, valspodar and zosuquidar (26,27), and the mixed inhibitor of P-gp and Bcrp1, elacridar (28). We also compared these results with those previously obtained by *in situ* brain perfusion, as both study designs were very similar.

MATERIALS AND METHODS

Chemicals and Reagents

[^{14}C]Imatinib (1.94 GBq/mmol), unlabeled imatinib (both as mesylate salts), valspodar, zosuquidar and elacridar were kindly provided by Novartis Pharma AG (Basel Switzerland). All other chemicals were of analytical grade. All doses or concentrations refer to the base form of compounds.

Animals

All experiments were performed on adult male FVB wild-type mice (Charles River, L'Arbresles, France), adult male FVB *Mdr1a/1b(−/−)* or *Bcrp1(−/−)* mice (Taconic Europe, Ry, Denmark), weighing 20 to 30 g. The mice were kept under standard conditions of temperature and lighting with free access to food and water. These studies complied with the Swiss Federal Act on Animal Protection (revised 2003) and with the Swiss Animal Protection Ordinance (revised 2001).

[1] Bihorel S, Camenisch G, Lemaire M, *et al.* Influence of Breast Cancer Resistance Protein (Abcg2) and P-glycoprotein (Abcb1a/1b) on imatinib mesylate (Gleevec®) transport across the mouse blood–brain barrier. Submitted for publication.

Blood and Brain Disposition of Imatinib

Each mouse received one of the efflux transporter inhibitors or the vehicle (controls) followed by [^{14}C]imatinib (approximately 7.5 MBq/kg). Imatinib was dissolved in 5% glucose; valspodar, zosuquidar and elacridar were dissolved in 2:6:2 mixture of ethanol:polyethylene glycol 200:5% glucose. The mice were anesthetized by inhalation of isoflurane (Abbott AG, Baar, Switzerland) before being given two intravenous injections, one in each surgically exposed saphenous vein. The first injection consisted of 3 mL/kg vehicle, valspodar, zosuquidar or elacridar, the second of 5 mL/kg imatinib (12.5 mg/kg). The time between the two injections did not exceed 2 min. Wild-type mice were given three doses of each inhibitor (3, 10 or 30 mg/kg), but *Mdr1a/1b(−/−)* mice were given only 10 mg/kg. The influence of these transporter inhibitors was not evaluated in *Bcrp1(−/−)* mice.

The *Mdr1a/1b(−/−)* mice were given 10 mg/kg because of the adverse effects 30 mg/kg inhibitor had on some wild-type mice. These animals took longer to recover after isoflurane anesthesia and had slowly reversible behavior alterations. This was most pronounced in the mice given valspodar, which causes cerebellar ataxia in rodents and humans (27,29). *Mdr1a/1b(−/−)* mice were given a medium dose of valspodar, zosuquidar and elacridar, as this produced limited apparent side-effects and a maximal increase in imatinib brain penetration in wild-type animals (see below).

The mice were sacrificed 1 h post-dose ($n = 4$ per group) as the brain distribution equilibrium of imatinib is reached at this time (7). Blood (~1 mL) was collected by puncture of the *vena cava*. The brain was sampled and quickly cleaned to remove the largest blood vessels. Blood and brain samples were stored at −20°C pending analysis.

Analytical Methods

Brain samples were homogenized in 3 mL demineralized water just before analysis. The radioactivity of the ^{14}C label in blood and brain homogenates was counted by liquid scintillation counting using a Tricarb 2500TR Liquid Scintillation Analyzer (Packard, Meriden, CT).

The concentrations of unchanged imatinib in blood and brain were determined by liquid chromatography. Unlabeled imatinib (2.5 μg) was added to each sample as an internal standard (100 μL stock solution). Blood samples (200 μL) were mixed with 100 μL water, 100 μL concentrated pH 12 buffer and 4 mL tert-butyl methylether. Brain samples (500 μL of homogenate) were treated with 500 μL acetonitrile, and centrifuged (13,000×*g*, 4 min at room temperature). The resulting supernatant was mixed with 250 μL pH 12 buffer and 4 mL tert-butyl methylether. All blood or brain samples were then shaken for 15 min and centrifuged (6,000×*g*, 10 min at room temperature). The organic layer was separated and evaporated to dryness in a vacuum centrifuge. The residues were reconstituted in 250 μL water:acetonitrile:formic acid (499:499:2 v/v/v) plus 75 μL n-hexane and centrifuged (13,000×*g*, 4 min at room temperature). The top hexane layer was removed and discarded. Aliquots of the remainder (150–200 μL) were injected onto a liquid chromatography system (MT2, Kontron Instruments, Zurich, Switzerland). Imatinib was separated from any metabolites and endogenous com-

pounds on an analytical Symmetry C-18 column, 3.5 μM, 150 × 4.6 mm (Waters AG, Rupperswil, Switzerland) equipped with a Symmetry C-18 guard column, 5 μM, 20 × 3.9 mm (Waters AG). The column was equilibrated with 98% mobile phase A (water:formic acid 998:2 v/v) and 2% mobile phase B (acetonitrile:formic acid 998:2 v/v) at 0.7 mL/min and the temperature was maintained at 30°C. Imatinib was eluted with a gradient of mobile phase B (2 to 26.5% in 20 min). The column was washed at the end of each run with 98% mobile phase B and 2% mobile phase A for 5 min, then equilibrated back to the initial conditions in 10 min.

The absorbance of imatinib was monitored using an ultraviolet detector set at 270 nm. The peak corresponding to the unchanged compound was collected in a polyethylene vial and analyzed for radioactivity by liquid scintillation counting. The concentration of unchanged imatinib in each sample was calculated from the ratio of the radioactivity in the eluate fraction and the peak area of ultraviolet absorbance of the unlabeled imatinib internal standard (30). The limits of quantification were 41 pmol/mL in blood and 79 pmol/g in brain.

Calculation of Pharmacokinetic Parameters for Imatinib and its Radioactive Metabolites

The presence of drug in brain vasculature must be considered when brain concentrations are measured in whole brain homogenates. A significant part of the drug found in brain homogenates may come from this vascular contamination, especially for any drug with a poor brain distribution. It must be excluded in order to assess accurately the penetration of the drug into the brain (31). We therefore subtracted the concentrations of unchanged imatinib or imatinib-related radioactivity in the vascular space (1.4% of the blood concentration 1 h post-dose) from the brain concentrations found in whole brain homogenates (7). The brain penetration of imatinib was estimated by the brain-to-blood concentration ratios (BB ratios) using these corrected brain concentrations.

The concentrations of radioactive metabolites of imatinib in blood and brain (expressed in μmol-radioequivalents per unit of volume or mass) were determined by calculating the differences between the concentrations of imatinib-related radioactivity and unchanged imatinib. The brain penetration of these radioactive metabolites was estimated by BB ratios. Lastly, the unchanged fractions in blood and in brain were calculated as the ratio of the concentrations of unchanged imatinib and imatinib-related radioactivity.

Statistical Analysis

All values are means ± standard deviation (SD) for four mice. The concentrations and BB ratios were compared by one-way analyses of variance on log-transformed values followed by multiple comparisons using Tukey's tests (SigmaStat 3.11, SPSS Inc, Chicago, IL). Three successive analyses were done for each parameter: the first compared the ten groups of wild-type mice, the second the four groups of *Mdr1a/1b(−/−)* mice and the last compared the control groups of wild-type, *Mdr1a/1b(−/−)* and *Bcrp1(−/−)* mice. Statistical significance was set at $p < 0.05$. Linear and non-linear regressions were performed with Graphpad Prism 4 (GraphPad Software Inc, San Diego, CA).

RESULTS

Influence of Valspodar and Zosuquidar on the Blood and Brain Concentrations of Imatinib

Both P-gp inhibitors had a dose-dependent influence on the blood concentrations of imatinib in wild-type mice (Fig. 1a). The first significant effects were observed with 10 mg/kg valspodar (2.2-fold increase) and zosuquidar (2.0-fold), but significantly higher elevations of blood concentrations were found at 30 mg/kg (3.3-fold increase for valspodar and 3.8-fold increase for zosuquidar). The brain penetration of imatinib, assessed by brain-to-blood concentration ratios (BB ratios), was rather low in wild-type mice (0.09 ± 0.01; Fig. 1b), but was significantly enhanced by valspodar and zosuquidar (Fig. 1b). The increases in the imatinib BB ratio were independent of the inhibitor dose within the range 3 to 30 mg/kg ($p > 0.05$ in all post-hoc comparisons) and the mean imatinib BB ratios produced by valspodar (0.35 ± 0.07, 4.1-fold increase) and zosuquidar (0.32 ± 0.09, 3.8-fold increase) were not statistically different.

The BB ratio of imatinib in the control group of *Mdr1a/1b(−/−)* mice was 6.4-fold higher than in the control group of wild-type mice, even though the blood concentrations of unchanged imatinib in the two groups were similar (Fig. 1a and b). Both valspodar and zosuquidar increased the blood concentration of imatinib in *Mdr1a/1b(−/−)* mice, but this impact was statistically significant only in the mice treated with zosuquidar (Fig. 1a). Valspodar (0.7-fold) and zosuquidar (0.5-fold) both tended to decrease the BB ratio of imatinib in these mice; again, the difference was statistically significant only in the mice given zosuquidar.

Influence of Elacridar on the Blood and Brain Concentrations of Imatinib

Like valspodar and zosuquidar, elacridar produced a dose-dependant increase in the blood concentrations of imatinib in wild-type mice; the increase (1.8-fold) in blood imatinib was significant with a dose of 3 mg/kg. The greatest change was produced by 30 mg/kg (4.4-fold increase, Fig. 1a). This inhibitor of both P-gp and Bcrp1 significantly enhanced the BB ratio of imatinib in wild-type mice (Fig. 1b). Its effect was not dose-dependant between 3 to 30 mg/kg ($p > 0.05$ in all post-hoc comparisons) and the mean imatinib BB ratio was 0.80 ± 0.16 (9.3-fold increase, Fig. 1b). Elacridar produced a significantly greater increase in the BB ratio of imatinib than did valspodar or zosuquidar, regardless of the dose ($p < 0.001$ in all post-hoc comparisons).

The blood concentration or brain penetration of imatinib was not significant changed in *Bcrp1(−/−)* mice given imatinib alone (Fig. 1a and b). In contrast, elacridar significantly increased the blood concentration of imatinib (3.2-fold) in *Mdr1a/1b(−/−)* mice, compared to the control knockout mice. Elacridar caused a small, statistically insignificant increase in BB ratio in this group of mice (1.2-fold, Fig. 1b).

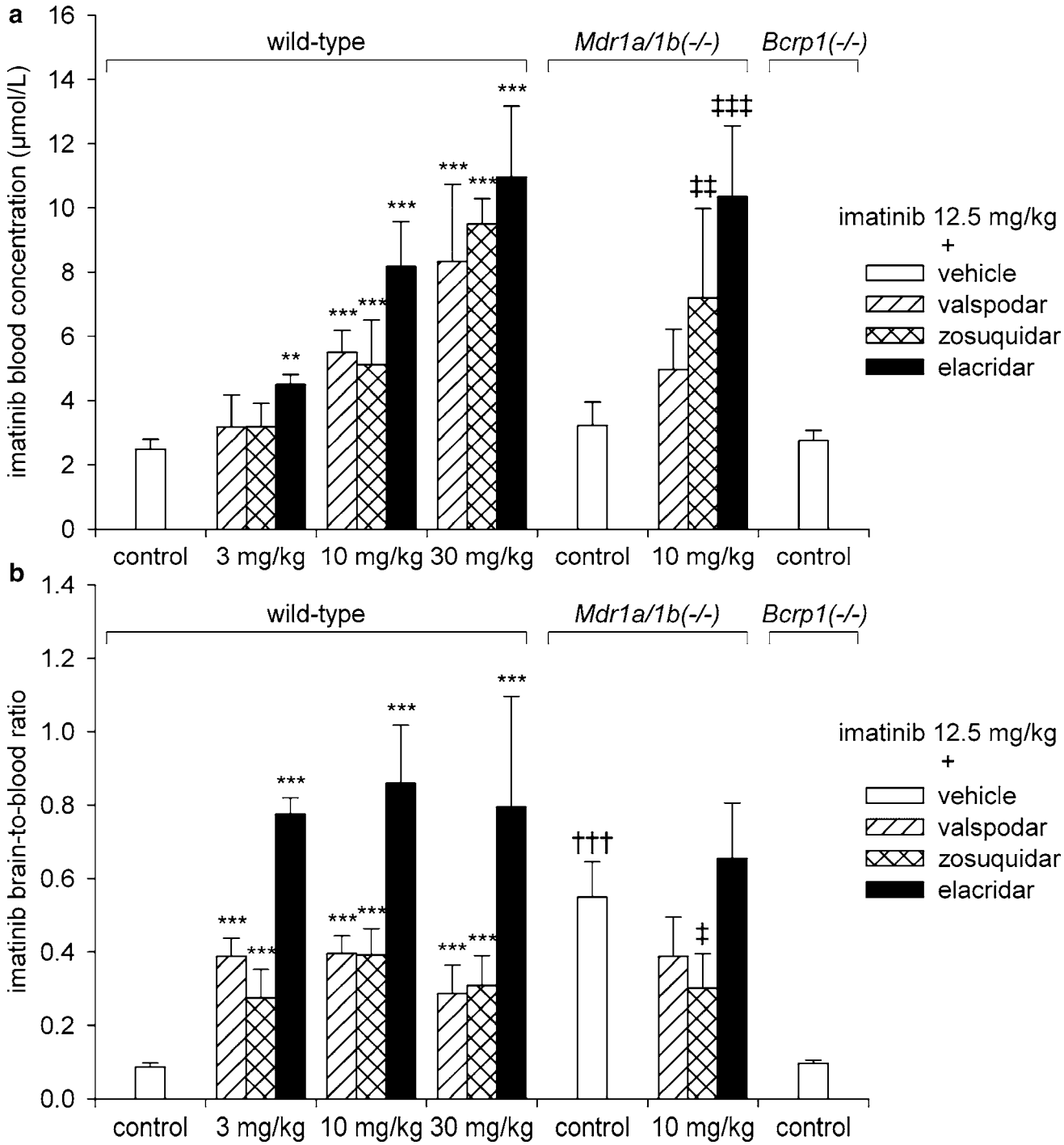

Fig. 1. Imatinib blood concentrations (µmol/L, **a**) and brain-to-blood concentration ratios (**b**) obtained 1 h after intravenous injection of imatinib (12.5 mg/kg) in FVB male wild-type, *Mdr1a/1b(−/−)* or *Bcrp1(−/−)* mice. Imatinib was administered after a first intravenous injection of either a mixture of ethanol:polyethylene glycol 200:5% glucose 2:6:2 v/v/v ("vehicle"), valspodar, zosuquidar or elacridar dissolved in vehicle (3, 10 or 30 mg/kg). *Columns*, means; *bars*, SD for four animals. *Asterisk*, control versus valspodar, zosuquidar or elacridar in wild-type mice; *double dagger*, control versus valspodar, zosuquidar or elacridar in *Mdr1a/1b(−/−)* mice; *dagger*, control wild-type mice versus control *Mdr1a/1b(−/−)* or *Bcrp1(−/−)* mice ($p < 0.05$, *one sign*; $p < 0.01$, *two signs*, $p < 0.001$, *three signs*).

Influence of Valspodar and Zosuquidar on the Blood and Brain Concentrations of Imatinib Radioactive Metabolites

The fractions of unchanged imatinib in the blood of the three control groups were comparable and reached 25–32% of the total radioactivity 1 h post-dose. These values are in line with previously reported data on imatinib metabolism in similar strains of mice (7).

The co-administration of valspodar with imatinib significantly increased the blood concentration of imatinib radioactive metabolites in a dose-dependent manner in wild-type

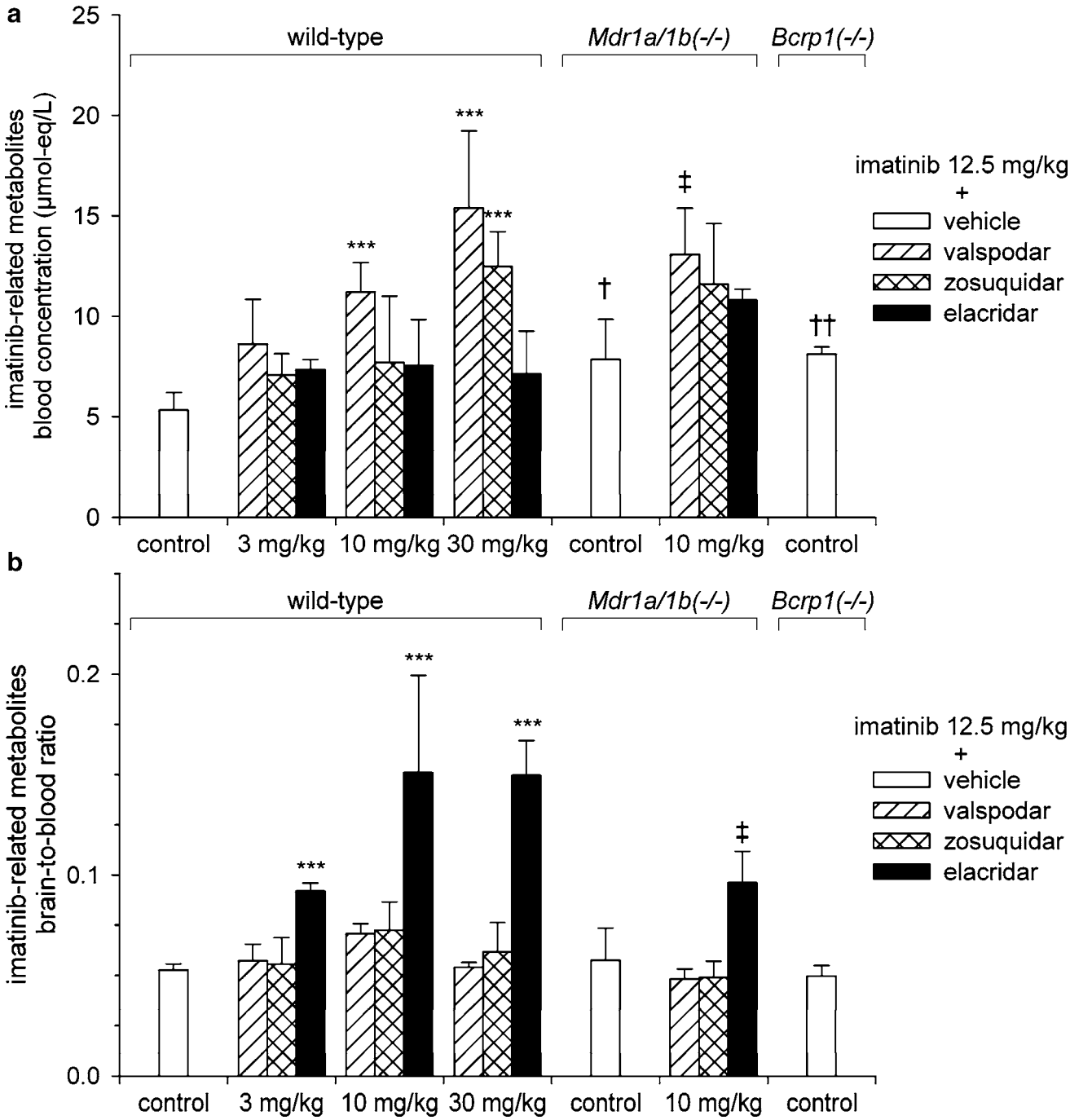

Fig. 2. Blood concentrations of imatinib radioactive metabolites (µmol-radioequivalent/L, **a**) and brain-to-blood concentration ratios (**b**) obtained 1 h after intravenous injection of imatinib (12.5 mg/kg) in FVB male wild-type, *Mdr1a/1b(−/−)* or *Bcrp1(−/−)* mice. Imatinib was administered after a first intravenous injection of either a mixture of ethanol:polyethylene glycol 200:5% glucose 2:6:2 v/v/v ("vehicle"), valspodar, zosuquidar or elacridar dissolved in vehicle (3, 10 or 30 mg/kg). *Columns*, means; *bars*, SD for four animals. *Asterisk*, control versus valspodar, zosuquidar or elacridar in wild-type mice; *double dagger*, control versus valspodar, zosuquidar or elacridar in *Mdr1a/1b(−/−)* mice; *dagger*, control wild-type mice versus control *Mdr1a/1b(−/−)* or *Bcrp1(−/−)* mice ($p < 0.05$, *one sign*; $p < 0.01$, *two signs*, $p < 0.001$, *three signs*).

mice (Fig. 2a). The greatest effect was observed at 30 mg/kg (2.9-fold increase). Zosuquidar had less effect on the blood concentrations of imatinib radioactive metabolites; a significant increase (2.3-fold) was obtained only with the highest dose (30 mg/kg). The brain penetration of imatinib radioactive metabolites was very low in wild-type mice; for instance, the BB ratio (0.05 ± 0.003) in the control group was half that obtained with the parent drug (Fig. 2b), resulting in a higher unchanged fraction in the brain (43%) than in the blood (32%). The changes in the brain concentrations of imatinib radioactive metabolites closely followed those of the blood concentrations in all groups of mice treated with valspodar or zosuquidar, resulting in insignificant changes in the BB ratios (Fig. 2b).

The BB ratios in both control groups of *Mdr1a/1b(−/−)* and wild-type mice were also similar, despite a significant increase

(1.5-fold) in the blood concentration of imatinib radioactive metabolites. The increases in the blood concentrations in *Mdr1a/1b(−/−)* mice produced by the transporter inhibitors were small (Fig. 2a). Given the variability associated with the experimental results, statistically significant differences were obtained only for the *Mdr1a/1b(−/−)* mice given valspodar (note the medium power of this statistical test, i.e. 0.678). Finally, valspodar and zosuquidar had no significant influence on the brain penetration of imatinib radioactive metabolites in *Mdr1a/1b(−/−)* mice (Fig. 2b).

Influence of Elacridar on the Blood and Brain Concentrations of Imatinib Radioactive Metabolites

Elacridar had no significant impact on the blood concentration of imatinib radioactive metabolites in wild-type mice, but enhanced their brain penetration in a dose-dependent manner with a plateau over 10 mg/kg (2.8-fold increase, $p < 0.001$; Fig. 2a and b).

Elacridar produced no significant change in the BB ratio of imatinib radioactive metabolites in *Bcrp1(−/−)* mice, despite a 1.6-fold increase in blood imatinib concentrations (Fig. 2a and b). In contrast, elacridar significantly increased the brain penetration of imatinib radioactive metabolites (1.7-fold) in *Mdr1a/1b(−/−)* mice, but not their blood concentration (see the remark on the medium statistical power in the previous paragraph).

DISCUSSION

We have measured the influence of two P-gp inhibitors, valspodar and zosuquidar, and one mixed inhibitor of P-gp and Bcrp1, elacridar on the concentrations of imatinib and its metabolites in the blood and brains of mice. The metabolites accounted for about 75% of the total radioactivity in the blood, hence it can lead to erroneous interpretations of inhibitors effects, if conclusions are based on measurements of total radioactivity (7,21)

Elacridar produced the greatest increases in the brain penetration of imatinib and its metabolites and has the most potential for increasing the clinical efficacy of imatinib against glioblastoma. Elacridar multiplied the brain penetration of imatinib 9-fold and of its metabolites 3-fold in wild-type mice, whereas valspodar and zosuquidar increased the brain penetration of imatinib by a factor 4 and had no effect on the metabolite BB ratio (Figs. 1b and 2b). This may be because elacridar inhibits both P-gp and Bcrp1 at the BBB (28), while valspodar and zosuquidar do not inhibit Bcrp1 (26,27). However, the increase in the brain distribution of imatinib and its metabolites can also result from an increase in their blood concentrations which favors the saturation of active transport proteins at the BBB (32).

Increases in the blood concentrations of antiproliferative drugs due to drug–drug interactions have been frequently reported as the major limitation for the clinical development of efflux transport inhibitors as reversal agents of multidrug resistance, primarily because these increases can lead to adverse or toxic effects. Nevertheless, the lowest tested doses (3 mg/kg) of the three inhibitors produced maximal increases in the imatinib BB ratio without any major increase in the blood concentrations, suggesting that this dose could markedly influence the brain distribution of imatinib while having limited risks of peripheral toxicity. As information on the pharmacokinetics of the transporter inhibitors in mice (except for their ability to inhibit efflux transporters) is lacking, additional studies are required to determine how these compounds modify the blood pharmacokinetics of imatinib, whether they act on its hepatic metabolism and elimination pathways, plasma protein binding or global tissue distribution. Nevertheless, the imatinib blood concentrations in the three control groups were similar, suggesting that systemic elimination is not decreased by inhibiting renal, hepatic or intestinal P-gp and/or Bcrp1. There was also no reduction in the concentrations of imatinib-related metabolites in the blood of mice treated with the inhibitors (Fig. 2a). This does not point to an inhibition of imatinib metabolism by the transporter inhibitors.

There were also differences in the blood concentrations of imatinib metabolites, particularly between the three control groups (Fig. 2a). The deficiency/inhibition of P-gp has been reported to influence the extent of degradation of CYP3A substrates by enhancing their intracellular availability in hepatocytes (33,34). As imatinib is degraded in the liver by CYP3A4 (35), the formation of its metabolites could be enhanced in knockout mice. Breedveld *et al.* also reported a reduced clearance of radioactive entities in *Mdr1a/1b(−/−)* and *Bcrp (−/−)* mice given intravenous [^{14}C]imatinib, suggesting a reduced elimination of imatinib and/or its radioactive metabolites (21). Once again, our study is limited and more complex investigations with multiple time-point pharmacokinetics or mass balance studies should be performed to identify the altered processes in the pharmacokinetics of imatinib and its metabolites in P-gp and/or Bcrp1 deficient mice.

The effects of valspodar, zosuquidar and elacridar on the blood concentrations of imatinib were very unlikely to saturate the active efflux of imatinib at the BBB for three reasons. First, the increase in the BB ratios of imatinib at low dose of valspodar and zosuquidar (3 mg/kg) was not related to any change in its blood concentration. Second, higher doses of both inhibitors did not increase the brain penetration of imatinib despite having a dose-dependent effect on its blood concentration. Finally, although 3 mg/kg elacridar significantly increased the blood concentration of imatinib, these levels were still lower than those found in mice given 30 mg/kg valspodar or zosuquidar, whereas the BB ratios were much higher in the former group than in the two latter groups. Likewise, the increases in the blood concentrations of imatinib in knockout mice were not associated with any increase in brain penetration. Thus, these results suggest that the influence of transporter inhibitors on the brain penetration of imatinib is probably due to their direct action on the efflux of imatinib at the BBB rather than an increase in its blood concentration. This also holds true for the radioactive metabolites of imatinib, as the changes in the BB ratios were not associated with any change in the blood concentrations (and vice versa).

This study also shows that the efflux of imatinib at the brain barriers involves P-gp, which plays a prominent role, and Bcrp1, which probably has an additive influence. Both P-gp inhibitors, valspodar and zosuquidar, considerably increased the brain penetration of imatinib in wild-type mice, as did the

defect of P-gp in *Mdr1a/1b(−/−)* mice, showing the impact of P-gp on imatinib uptake by the brain. The influence of Bcrp1 is less clear. Elacridar had a greater effect on the brain penetration of imatinib in wild-type mice than did either valspodar or zosuquidar, suggesting that Bcrp1 mediates an additional efflux of imatinib at the BBB. However, *Mdr1a/1b(−/−)* mice given imatinib plus elacridar showed only a non-significant increase in imatinib brain penetration. This effect was significant when the more sensitive technique of *in situ* brain perfusion was used (refer to footnote 1). Lastly, the BB ratios of imatinib in *Bcrp1(−/−)* and wild-type mice were similar, which is consistent with our first study. We previously postulated that a functional P-gp at the *Bcrp1(−/−)* mouse BBB could keep the brain uptakes of imatinib in the two strains of mice similar (refer to footnote 1).

The changes in the imatinib BB ratio or net transport coefficient in *Mdr1a/1b(−/−)* mice treated with the transporter inhibitors found in our two studies are consistent, except that zosuquidar produced unexpected opposite effects (Fig. 1b, see footnote 1). Valspodar has been reported to produce a similar decrease in the brain distributions of the P-gp substrates digoxin, paclitaxel and nelfinavir in *Mdr1a/1b*(−/−) mice (36–38). It is not yet clear how valspodar and zosuquidar affect the brain uptake of imatinib in this particular strain of mice. They may inhibit/activate an active transporter (yet undefined and potentially specific to each inhibitor), or modify the diffusion properties of brain endothelial cell membranes, both of which could change the apparent drug permeability. In the light of the effect of both inhibitors on the P-gp-mediated efflux of imatinib in wild-type mice, it remains to be seen whether this effect is present in wild-type mice, or whether it is specific to *Mdr1a/1b(−/−)* mice and the up/down regulated expression of some transport protein.

Data from our *in situ* brain perfusion experiments (net transport coefficient K_{net}) correlate reasonably well with those obtained in the present study (BB ratios). Pairs of values were formed between parameters measured in the closest conditions with each method, i.e. imatinib with or without a transporter inhibitor in wild-type, *Mdr1a/1b(−/−)* or *Bcrp1(−/−)* mice (Fig. 3). The mean imatinib BB ratio in wild-type mice treated with one transporter inhibitor was associated with the maximal K_{net} obtained after perfusion of imatinib plus this inhibitor. All attempts at modeling the global data set with linear or non-linear relationships failed. However, fairly good linear correlations were found between the BB ratios and K_{net} obtained in wild-type ($R^2 = 0.968$) and *Mdr1a/1b(−/−)* mice considered separately ($R^2 = 0.999$, provided that the point relative to imatinib-zosuquidar co-administration is excluded from the regression; $R^2 = 0.017$ if not). This double correlation supports previous results showing that the permeability of imatinib across the BBB in wild-type and in knockout mice is different, regardless of the deficiency or the blockade of P-gp and Bcrp1 (refer to footnote 1). We postulated a change in the properties of the plasma membrane of brain endothelial cells in knockout mice or an up-regulation in the expression of influx transport proteins. Therefore, conclusions should be carefully drawn from direct comparisons of brain penetrations measured in wild-type and *Mdr1a/1b(−/−)* mice, as a significant difference may be due not only to a deficiency of P-gp, but also to changes in the BBB properties. In the current study, the amplitude of this difference combined with the

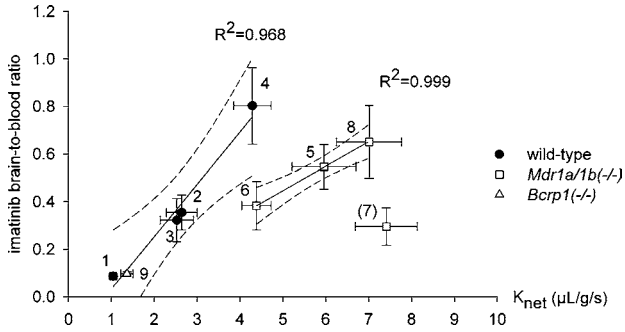

Fig. 3. Correlation between imatinib brain-to-blood concentration ratios obtained 1 h after intravenous injection of 12.5 mg/kg imatinib and brain transport coefficient (K_{net}) obtained by *in situ* brain perfusion of 0.5 µM imatinib in similar conditions: wild-type mice given imatinib alone (1) or with valspodar (2), zosuquidar (3) or elacridar (4); *Mdr1a/1b(−/−)* mice given imatinib alone (5) or with valspodar (6), zosuquidar (7) or elacridar (8); *Bcrp1(−/−)* mice given only imatinib (9). Linear regressions were calculated separately for wild-type and *Mdr1a/1b(−/−)* mouse related values (point 7 was excluded from the regression). The two best-fit models are shown by the solid lines while the 95% confidence intervals are represented by the dotted lines. Data partially taken from Bihorel *et al.* (submitted for publication). *Points*, means; *bars*, SD for four to five animals.

results obtained with P-gp inhibitors in wild-type mice indicate that it is due to the lack of P-gp.

Finally, we also examined the brain penetration of radioactive metabolites of imatinib. The permeability of these metabolites across the BBB was neither modified by valspodar or zosuquidar nor by the single deficiency of P-gp or Bcrp1 in knockout mice. The brain penetration of imatinib-related metabolites was enhanced only by elacridar in both wild-type and *Mdr1a/1b(−/−)* mice. These results suggest that the transport activities of P-gp and Bcrp1 must be concomitantly altered to significantly enhance the brain uptake of radioactive metabolite(s) of imatinib in mice. The pharmacological activity of the main metabolite of imatinib in humans, CGP74588, is similar to that of imatinib (8, 39). *In situ* brain perfusion studies showed that CGP74588 is most likely a Bcrp1 substrate, as the brain penetration of this metabolite is enhanced in wild-type animals co-perfused with elacridar and in *Bcrp1(−/−)* mice (refer to footnote 1). We therefore expected CGP74588 to contribute to an increase in the brain penetration of radioactive metabolites in *Bcrp1(−/−)* mice. However, recent investigations have revealed that the metabolism of imatinib in mice and humans is quantitatively different. Mice given an oral dose of 200 mg/kg [^{14}C]imatinib had only a minor proportion of the radioactivity in the plasma that corresponded to CGP74588, suggesting that it is only a minor metabolite in mice.[2] This may explain why the brain penetration of imatinib radioactive metabolites in wild-type and *Bcrp1(−/−)* mice is similar. It is currently unknown which metabolite or metabolites of imatinib are responsible for the enhanced brain penetration of imatinib radioactive metabolites in mice given elacridar. Additional studies are needed to identify these compounds and understand the clinical implications of giving elacridar and imatinib together.

[2] Wiegand H and Pfaar U, unpublished data

In summary, this study shows that P-gp, and probably Bcrp1, modulate the brain transport of imatinib and its metabolites, although in different manners. Mice given imatinib together with valspodar, zosuquidar or elacridar also had significantly increased the brain penetration of imatinib, whilst only elacridar enhanced the penetration of imatinib metabolites present in mouse blood. This effect was probably due to the inhibition of the P-gp and Bcrp1-mediated efflux at the brain barriers. Our findings, together with our *in situ* brain perfusion experiments, suggest that giving imatinib with efflux transporter inhibitors, especially elacridar, enhances the delivery of both imatinib and CGP74588 to the brain. Whether this kind of combination can improve the antitumor action of imatinib in patients with MG remains to be seen.

ACKNOWLEDGMENTS

We thank Dr. Yves Auberson (Novartis Institutes for Biomedical Research) for providing zosuquidar and elacridar, Dr. Rachael Profit and Dr Owen Parkes for editing the English text. This work was supported by Novartis Pharma AG contract (Novartis-INSERM n° 03035A10) to Dr. Sébastien Bihorel and Dr. Jean-Michel Scherrmann.

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