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Blood-brain barrier penetration of the enantiomers of venlafaxine and its metabolites in mice lacking P-glycoprotein

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Abstract

According to *in vitro* studies the enantiomers of venlafaxine display different degrees of serotonin and noradrenaline reuptake inhibition. Therefore, clarification of the enantiomeric drug distribution between serum and brain is highly warranted. To elucidate if P-glycoprotein (P-gp) in a stereoselective manner transports venlafaxine and its metabolites out of the brain we used *abcb1ab* double-knockout mice that do not express P-gp. A single dose of racemic venlafaxine (10 mg/kg bw) was intraperitoneally injected to knockout (-/-) and wildtype (+/+) mice. Serum and brain samples were collected 1, 3, 6 and 9 h following drug administration for analysis by LC/MS/MS. One to six hours post-dose, the brain concentrations of venlafaxine, O-desmethylvenlafaxine and N-desmethylvenlafaxine were 2-3, 2-6 and 3-12 times higher in *abcb1ab* (-/-) mice compared to *abcb1ab* (+/+) mice, respectively. No major differences in the serum and brain disposition of the S- and R-enantiomers of venlafaxine and its metabolites were found between the groups. We conclude that P-gp decreases the penetration of the S- and R-enantiomers of venlafaxine and its major metabolites into the brain. No evidence of a stereoselective P-gp mediated transport of these substances was observed.

KEYWORDS: *abcb1ab*, blood-brain barrier, enantiomer, knockout mice, P-glycoprotein, venlafaxine

1. Introduction

The passage of compounds from the systemic blood into the central nervous system (CNS) is limited by the presence of the capillary endothelial cells of the blood-brain barrier (BBB).

Two important functions of the BBB are the protection of the brain against potentially toxic substances and the maintenance of a constant internal environment, which is of primary importance to the optimal neuronal functioning of the brain. The barrier has cellular tight junctions and express transport systems which serve active passage into the brain, and active efflux out of the brain (Ballabh et al., 2004).

One of the most studied transport proteins is P-glycoprotein (P-gp) which is a product of the multidrug resistance (MDR) gene *MDR1* (also known as *ABCB1*). P-gp is a membrane-bound ATP-dependent efflux transporter present at high concentrations in the apical membrane of the endothelial cells of the brain capillaries (Beaulieu et al., 1997; Biegel et al., 1995). P-gp and other drug transporters have significant effects on absorption, distribution, metabolism and excretion of drugs. These effects are especially apparent when oral bioavailability and the CNS distribution of several drugs are considered (Fromm, 2000). This is even more important as no structural similarity between the P-gp substrates has been found that would allow predicting new substances that may be P-gp substrates. The P-gp substrates include anticancer agents, immunosuppressants, steroid hormones, antimicrobial agents, opioids and CNS active drugs (Fromm, 2002).

The prescribing of antipsychotic and antidepressant drugs has increased appreciably during the past decade (Pirraglia et al., 2003; Sonnenberg et al., 2008). One example of such a drug is the antidepressant venlafaxine, a potent serotonin and noradrenalin reuptake inhibitor with some effect also on dopamine reuptake (Ellingrod and Perry, 1994). Venlafaxine is a racemic

mixture of the (–)-R-enantiomer and (+)-S-enantiomer. The R-enantiomer inhibits both serotonin and noradrenaline reuptake *in vitro*, while the S-enantiomer inhibits only serotonin reuptake (Holliday and Benfield, 1995; Muth et al., 1986). Venlafaxine undergoes extensive first-pass metabolism by cytochrome P450 (CYP) enzymes into its major active metabolite O-desmethylvenlafaxine (Odm-venlafaxine). Two minor metabolites are N-desmethylvenlafaxine (Ndm-venlafaxine) and N,O-didesmethylvenlafaxine (Ddm-venlafaxine). Odm-venlafaxine inhibits reuptake of serotonin and noradrenaline with similar potency to that of venlafaxine, but no data are available on the relative efficiency of the two enantiomers. Ndm-venlafaxine and Ddm-venlafaxine may also be considered as pharmacologically active, but they are claimed to be less potent than the parent drug (Muth et al., 1986; Muth, 1991).

Between individuals the results of drug therapy can vary depending on the efficiency of the P-gp *ABCB1* gene owing to various polymorphisms among individuals leading to different drug levels and therapy outcome (Linnet and Ejlsing, 2008; Uhr et al., 2008). Consequences of drug efflux transporters, like P-gp, in the BBB include limiting the distribution of substances that are beneficial to treat psychiatric disorders, and increasing the problem of drug-drug interactions and potential toxicity. The importance of P-gp in the BBB has been studied extensively in cultured mammalian brain capillary endothelial cells (Tsuji et al., 1992). However, the *in vitro* models cannot completely represent the complex *in vivo* situation. To overcome this problem, transgenic mice can be used. In humans, P-gp is encoded by a single *ABCB1* gene, whereas two genes, *abcb1a* and *abcb1b*, encode P-gp in mice (Devault and Gros, 1990). The *abcb1ab* knockout mouse was developed to investigate the role of P-gp *in vivo* and has provided a unique and valuable pharmacological tool (Lagas et al., 2009; Schinkel et al., 1997; Schinkel et al., 1994). This animal model shows that venlafaxine is a P-

gp substrate (Doran et al., 2005; Uhr et al., 2003). However, no studies have examined the role of P-gp on possible stereoselective differences in the disposition of venlafaxine and its three main metabolites in the brain. Such knowledge is important since the enantiomers of venlafaxine display different pharmacological activities *in vitro*. Whether Odm-venlafaxine, Ndm-venlafaxine and Ddm-venlafaxine are P-gp substrates has not previously been reported. Therefore, the aim of this study was to determine whether the brain uptake of the S- and R-enantiomers of venlafaxine, Odm-venlafaxine, Ndm-venlafaxine and Ddm-venlafaxine differs between *abcb1ab* knockout mice and corresponding wildtype mice.

2. Experimental procedures

2.1. Animals

All experiments were conducted in accordance with the European guidelines for care and use of laboratory animals and approved by local authorities. Male *abcb1ab* (-/-) mice (31-35 g) on a FVB/N background (Taconic, Germantown, NY) and male FVB/N wildtype mice (*abcb1ab* (+/+)) (31-33 g) were housed in groups of 3-5 in cages with sawdust bedding under normal indoor temperature and humidity, and maintained on 12-hour light-dark cycle, with food and water *ad libitum*.

2.2. Drugs and Chemicals

Racemic venlafaxine-HCl (kindly provided by Wyeth-Ayerst Research, Rouses Point, VT, USA) was dissolved in 0.9% NaCl and administered intraperitoneally (i.p.). All other chemicals were of the purest grade commercially available.

2.3. Experimental design

Venlafaxine was administered to *abcb1ab* (-/-) knockout (n=10 per time point) and *abcb1ab* (+/+) FVB/N wildtype (n=10 per time point) mice by a single i.p. injection during a brief anesthesia with isoflurane (Forene®, Abbott GmbH & Co. KG, Wiesbaden, Germany). The

concentration used was 10 mg/kg body weight and the total volume injected was 1 mL. At four different time points (1, 3, 6 and 9 hours) following drug administration, the mice were anesthetized with isoflurane and decapitated. Trunk blood was collected in tubes and thereafter the brain was removed. The blood samples were left at room temperature to allow clotting and thereafter centrifuged (3000g for 5 min x 2). Serum was collected for the determination of the concentrations of the enantiomers of venlafaxine and its three main metabolites. All samples were frozen at -70°C until further analysis.

2.4. Drug extraction procedure

Venlafaxine and metabolites were extracted from serum and brain by a solid-phase extraction (SPE) procedure. The brain samples were weighed and homogenized in 2 mL ultra pure water using a sonifier (Sonics VibraCell VC 130; Chemical Instruments AB, Lidingö, Sweden) followed by centrifugation (2000g for 20 min). The Isolute C8 100 mg (International Sorbent Technology, Hengoed, UK) SPE column was conditioned with methanol and Milli-Q water. Thereafter, 0.2 mL of calibration standard, control, brain homogenate or serum sample, and 20 µL of the internal standard mexiletine (0.05 mmol/L), was added. After washing with 1 mL Milli-Q water, followed by 2 mL of 50% methanol and 2 mL of acetonitrile, the sample was eluted with 1.5 mL acetonitrile containing 10 mM trifluoroacetic acid. The eluate was evaporated with nitrogen at 50°C in a block thermostat (Grant QBT2; Grant Instruments (Cambridge) Ltd, UK) and reconstituted in 50 µL of mobile phase (10:90 v/v; 10 mM tetrahydrofuran: ammonium acetat, pH 6.0) and transferred to a vial.

2.5. Drug concentration determination

The concentrations of the S- and R-enantiomers of venlafaxine and its metabolites in serum (nmol/L) and brain homogenates (pmol/g $\approx$ nmol/L) were determined by using an Acquity liquid chromatography (LC) system (Waters, Milford, MA, USA) and a Sciex API 4000 tandem mass detector equipped with an electrospray ionization (ESI) ion source (PE Sciex,

Ontario, Canada). A volume of 5 μ L reconstituted sample was injected onto a Chirobiotic-V column (5 μ m particle size, 250 x 2.1 mm; Astec, Basle, Switzerland) protected with an in-line filter (VICI AB International, Switzerland). The temperature of the column was set to 10°C using a Jones Chromatography Model 7955 column chiller/heater (Hengood, UK) and the flow rate of the mobile phase was 0.2 mL/min. Formic acid (0.05%) in acetonitrile was delivered through a Gynkotec 480 pump (Dionex; Sunnyvale, CA, USA) and added post column at a flow rate of 0.2 mL/min to increase the sensitivity. Data acquisition and peak integration, recording the area of the peaks, were performed using Analyst 1.4 software (PE Sciex; Ontario, Canada). Standards were prepared with the following concentrations 1, 2.5, 10, 25, 100, 250 and 1000 nmol/L (for each enantiomer of venlafaxine and Odm-venlafaxine) and 0.5, 1.25, 5, 12.5, 50, 125 and 500 nmol/L (for each enantiomer of Ndm-venlafaxine and Ddm-venlafaxine). Two different quality controls were prepared with concentrations of 2 and 500 nmol/L (for each enantiomer of venlafaxine and Odm-venlafaxine) and 1 and 250 nmol/L (for each enantiomer of Ndm-venlafaxine and Ddm-venlafaxine). The mean extraction recoveries for venlafaxine and its metabolites in serum ranged between 75-110%. The lower limit of quantification was 0.5 nmol/L for the enantiomers of venlafaxine and Odm-venlafaxine, and 0.25 nmol/L for Ndm-venlafaxine and Ddm-venlafaxine.

2.6. Statistical analysis

Results are expressed as means $\pm$ standard error of the means (SEM). A two-tailed Student's *t*-test for unpaired observations was used to compare differences between groups. P values <0.05 were considered statistically significant. All statistical analyses were performed using StatView[®] for Windows Version 5.0 (SAS[®] Institute Inc., Cary, NC; USA).

3. Results

3.1. Concentrations of the enantiomers of venlafaxine and metabolites in brain

The concentrations of the S- and R-enantiomers of venlafaxine and its metabolites are displayed in Figure 1. Table 1 shows the total (S+R) drug and metabolite concentrations as well as the concentration ratios between knockout and wildtype mice in serum and brain. In the brain, the S- and R-enantiomeric concentrations of venlafaxine were found to be 2-4 times higher in the *abcb1ab* knockout mice as compared to the wild type mice one, three and six hours following drug administration. No significant difference in venlafaxine concentrations was seen between the two groups nine hours post dose. For the main metabolite Odm-venlafaxine, the concentrations of the S- and R-enantiomers were 3-7 times higher in the knockout mice than in the wild type mice at all four time points ($p < 0.0001$). The enantiomeric S- and R-Ndm-venlafaxine levels were 3-12 times higher in knockout mice as compared to wild type mice ($p < 0.0001$ at all four time points). The concentrations of S-Ddm-venlafaxine were measurable one and three hours following drug administration in 90% and 50% of the *abcb1ab* knockout mice (concentration range 1.9-4.0 nmol/L and 1.6-2.2 nmol/L, respectively). R-Ddm-venlafaxine could only be measured after one hour in two out of ten of the *abcb1ab* knockout mice. The levels of Ddm-venlafaxine in the brain were below the limit of quantification for all the wildtype mice (data not shown).

3.2. Concentrations of the enantiomers of venlafaxine and metabolites in serum

One and three hours following drug administration, no significant differences were observed in the S- and R-enantiomeric concentrations of venlafaxine, Odm-venlafaxine and Ndm-venlafaxine in the knockout mice as compared to the wild type mice (exception for S-Odm-venlafaxine at 3 hours; Figure 1 and Table 1). The S- and R-concentrations of venlafaxine, Odm-venlafaxine and Ndm-venlafaxine were found to be about 2 times higher in the *abcb1ab*

knockout mice than in the wild type mice six hours following drug administration ($p < 0.001$, $p < 0.01$ and $p < 0.01$, respectively). The S- and R-Ddm-venlafaxine concentrations were around the same in the two groups one and three hours following drug administration (data not shown). The serum levels of Ddm-venlafaxine were below the limit of quantification for the two later time points in both the *abcb1ab* knockout mice and the wildtype mice.

3.3. S/R concentration ratios of venlafaxine and metabolites

The enantiomeric S/R concentration ratios of venlafaxine, Odm-venlafaxine and Ndm-venlafaxine in serum and brain at four different time points after drug administration in *abcb1ab* knockout mice and wildtype mice are shown in Table 2. For venlafaxine, the S/R ratios in brain were somewhat lower in P-gp deficient mice (~1.1-1.3) as compared to wildtype mice (~1.4-1.5) and this difference was statistically significant one, three and six hours following drug administration ($p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively). However, the knockout/wildtype ratios for the S- to R-enantiomers in brain were not different one, three and six hours post dose (2.1, 2.9 and 2.6 for S-venlafaxine vs. 2.7, 3.6 and 3.0 for R-venlafaxine, respectively). In serum, no significant differences in venlafaxine S/R ratios were observed between *abcb1ab* knockout mice and wild type mice. The S/R ratios of Odm-venlafaxine and Ndm-venlafaxine were not significantly different between *abcb1ab* knockout and wildtype mice in the brain with two exceptions (Odm-venlafaxine at the 9 hour time point and Ndm-venlafaxine at the 1 hour time point).

3.4. Brain/serum concentration ratios of venlafaxine and metabolites

The brain/serum concentration ratios of venlafaxine, Odm-venlafaxine and Ndm-venlafaxine are displayed in Figure 2. In mice lacking functional P-gp, the brain/serum ratio of venlafaxine were significantly higher compared to wild type mice at all four time points (1, 3, 6 and 9 h) after injection of racemic venlafaxine ($p < 0.01$, $p < 0.0001$, $p < 0.05$ and $p < 0.05$,

respectively). At the corresponding time points, the brain/serum concentration ratios of Odm-venlafaxine were found to be 3-4 times higher in the *abcb1ab* knockout mice as compared to the wild type mice (p-values ranging between $p < 0.01$ to $p < 0.0001$). One to nine hours post dose, the brain/serum concentrations ratios of Ndm-venlafaxine were higher in the *abcb1ab* knockout mice as compared to the wild type mice (p-values ranging between $p < 0.001$ to $p < 0.0001$).

3.5. Metabolite/Parent drug ratios in serum and brain

The metabolite/parent drug (M/P) ratios for Odm-venlafaxine/venlafaxine in serum were similar between the two groups at one, three and six hours post dose (0.12, 0.25 and 0.56 in knockout mice vs. 0.11, 0.25 and 0.75 in wildtype mice, respectively). In brain, the M/P ratios for Odm-venlafaxine/venlafaxine were 0.06, 0.54 and 2.4 in *abcb1ab* knockout mice and 0.05, 0.29 and 1.2 in wildtype mice (1 h, 3 h and 6 h post dose, respectively). The serum M/P ratios for Ndm-venlafaxine/venlafaxine were similar between knockout mice and wildtype mice (0.4-0.5 at 1 h, 0.8-1.0 at 3 h and 0.8-0.9 at 6 h post dose). The Ndm-venlafaxine/venlafaxine ratios in brain were 0.14, 1.8 and 8.6 in *abcb1ab* knockout mice and 0.09, 0.74 and 2.2 in wildtype mice (1 h, 3 h and 6 h post dose, respectively).

4. Discussion

We show for the first time data on the disposition of the enantiomers of venlafaxine and its metabolites in the P-gp knockout mouse model. The results of this study demonstrate that P-gp decreases the brain uptake of the S- and R-enantiomers of venlafaxine as well as the main metabolites (Odm-venlafaxine and Ndm-venlafaxine). This was confirmed from the observation of higher brain concentrations of venlafaxine and the metabolites Odm-venlafaxine and Ndm-venlafaxine in the *abcb1ab* (-/-) mice as compared to the *abcb1ab* (+/+) mice. Furthermore, we saw no evidence of a stereoselective P-gp mediated transport of

these substances over the BBB. This finding is important since it has been reported that the enantiomers of venlafaxine display different potencies of serotonin and noradrenaline reuptake inhibition *in vitro*. However, data on differential clinical properties of S- and R-venlafaxine are lacking.

In accordance with Uhr et al (2003), the brain concentration of venlafaxine was significantly higher in the knockout mice compared to wildtype mice. The present serum and brain knockout/wildtype ratios were 1.1 and 2.4, respectively, compared to 1.3 and 2.3 in the study by Uhr and co-workers (Uhr et al., 2003). However, in their study they measured total (i.e. the sum of the S- and R-enantiomers; S+R) concentrations of venlafaxine and one metabolite (Ndm-venlafaxine) one hour after s.c. administration of racemic venlafaxine. In our study, the separate enantiomers of venlafaxine, and its three main metabolites (Odm-venlafaxine, Ndm-venlafaxine and Ddm-venlafaxine) were measured at four different time points (1, 3, 6 and 9 hours after i.p. injection). The results in the present study are also in line with the results reported by Doran and co-workers although they investigated the total (S+R) concentration of venlafaxine (Doran et al., 2005). One hour after drug administration, the brain/serum concentrations of venlafaxine in knockout mice were 10 vs. 7.7 and the corresponding ratios in wildtype mice were 4.3 vs. 4.2 (present study vs. Doran et al., 2005). Thus the present results together with those from other laboratories confirm the validity of the *abcb1ab* knockout mouse model and its usefulness for the investigation of the function of BBB transporters in relation to the pharmacokinetics of different drugs.

So far several antidepressant and antipsychotic drugs have been determined to be P-gp substrates by the use of the *abcb1ab* knockout mice model. Some examples are doxepin, paroxetine, citalopram, fluvoxamine, sulpiride, risperidone, paliperidone and trimipramine

(Doran et al., 2005; Kirschbaum et al., 2008; Uhr and Grauer, 2003; Uhr et al., 2003) whereas mirtazapine and haloperidol have not been proved to be P-gp substrates (Kirschbaum et al., 2008; Uhr et al., 2003) in this animal model. Such an *in vivo* model has a clear advantage, since the BBB is very complex making it difficult to study interactions between the BBB and drugs *in vitro*. For example, studies in Caco-2 cells have given contradicting results and it is still unclear whether venlafaxine is a substrate or not *in vitro* (Ehret et al., 2007; Oganessian et al., 2009). Furthermore, using monolayers of bovine brain microvessel endothelial cells, Rochat and co-workers reported that citalopram crosses the BBB without the influence of P-gp (Rochat et al., 1999). By contrast, Uhr and Grauer (2003) used the *abcb1ab* knockout mouse model to show that citalopram is actively transported out of the brain by P-gp and similar results were found by Doran et al. (2005). *In vivo* models like the use of *abcb1ab* knockout mouse have an additional advantage, namely that they allow studies of pharmacokinetic interactions as well as pharmacodynamic effects (Kirschbaum et al., 2008). To this end, Schinkel and co-workers showed that complete loss of P-gp does not lead to any marked physiological abnormalities, and that the *abcb1ab* knockout mouse thus provide a good model to study the roles of P-gp in pharmacology and toxicology (Schinkel *et al.*, 1997).

In the present study, we also investigated the disposition of the S- and R-enantiomers of venlafaxine and its metabolites in serum and brain. The serum venlafaxine S/R concentration ratios were 1.2-1.4 in *abcb1ab* (-/-) mice and 1.1-1.3 in *abcb1ab* (+/+) mice, but this difference was not statistically significant. Similarly, no significant differences in S/R concentration ratios for Odm-venlafaxine or Ndm-venlafaxine were observed in serum or brain between knockout and wildtype mice. In contrast, significant differences in venlafaxine brain S/R concentration ratios were observed between knockout and wildtype mice with a somewhat lower S/R ratio in knockout mice. However, when comparing the

knockout/wildtype ratios for the S- to R-enantiomers in brain no differences in enantiomeric concentrations between the two groups were found. These data suggest that the P-gp affinity does not differ between the enantiomers of venlafaxine, Odm-venlafaxine or Ndm-venlafaxine. An absence of stereoselective P-gp mediated transport has also been found for R/S-verapamil (Luurtsema et al., 2003; Sandstrom et al., 1998). In contrast to our findings, a stereoselectivity for R/S-methadone in the *abcb1ab* (-/-) mouse model was reported in which the brain knockout/wildtype ratios of R- and S-methadone were 15.3 and 23.5, respectively (Wang et al., 2004). The pharmacokinetics of fexofenadine was also shown to display stereoselectivity with a higher P-gp affinity for the S-enantiomer than for the R-enantiomer (Miura et al., 2007). However, measuring the concentrations of enantiomers when steady-state conditions are reached might give results more closely related to the situation in humans, in which drugs are given over longer periods. This might be one limitation with the present study and more work is necessary to assess the role of P-gps in chiral discrimination.

Chiral considerations are relevant to various aspects of pharmacology and toxicology. Stereoselective metabolism of chiral drugs can additionally influence pharmacokinetics, pharmacodynamics and toxicity (Smith, 2009). To our knowledge, no published data in mice are available to compare with the present results. In dogs, the mean plasma S/R concentration ratios of venlafaxine were 1.5 (chronic administration of 22 mg/kg/day) and in rats 5.5 (chronic administration of 120 mg/kg/day) (Wang et al., 1992). In another rat model the S/R venlafaxine ratio in serum was 1.4 after chronic administration of racemic venlafaxine (Wikell et al., 2002). In humans, a mean plasma S/R enantiomeric ratio of 1.3 (n=9) was reported after a single 50 mg dose of racemic venlafaxine (Wang et al., 1992). Eap and co-workers published a case report with a patient displaying a serum S/R venlafaxine ratio of 1.9 (Eap et al., 2000). Gex-Fabry et al. investigated 35 patients on chronic medication with

venlafaxine and they reported S/R concentration ratios between 0.4-2.5 (median 1.1) (Gex-Fabry et al., 2004; Gex-Fabry et al., 2002). Ciusani and co-workers studied 5 patients receiving individually adapted doses between 75-225 mg/day and they found mean plasma S/R ratios of 1.16, 0.94 and 1.01 for venlafaxine, Odm-venlafaxine and Ndm-venlafaxine, respectively (Ciusani et al., 2004). Taken together, there seems to be both species differences and inter-individual variability in the S/R disposition that need to be considered when results from different studies are compared. One also has to consider how the drug was administered and the time of sampling in relation to the time when the drug was given.

In the present study, a stereoselective disposition of Odm-venlafaxine and Ndm-venlafaxine in both knockout mice and wildtype mice was found. This stereoselectivity is probably due to a stereoselective metabolism of venlafaxine by the CYP-enzymes. Venlafaxine is metabolized by CYP2D6 to its main metabolite Odm-venlafaxine and by CYP3A4 to Ndm-venlafaxine. The metabolite Ndm-venlafaxine is then further metabolized to Ddm-venlafaxine, possibly by CYP2D6 (Fogelman et al., 1999; Muth et al., 1986; Muth, 1991; Otton et al., 1996). Although CYP2D6 metabolizes both the S- and R-enantiomers of venlafaxine to Odm-venlafaxine it has been suggested that this enzyme is stereoselective. In humans, stereoselective metabolism of venlafaxine by CYP2D6 was reported but there was no evidence that this was the case for CYP3A4 (Eap et al., 2003).

Uhr and co-workers were the first to suggest a relationship between genetic variants in the *ABCB1* gene and the clinical efficacy of antidepressants, most likely by influencing their access to the brain (Uhr et al., 2008). Such an observation might apply to other medications as well. For example, it has recently been reported in a clinical study that *ABCB1* polymorphism influences the pharmacokinetics of the antipsychotic drug clozapine (Jaquenoud Sirot et al.,

2009). However, whether this finding correlates with the clinical response requires further studying. Nevertheless, the investigation of transporters like P-gp remains highly relevant especially as no structural similarity between the P-gp substrates exists. It is a necessity that therapeutic levels of drugs reach the brain in order to treat CNS disorders like major depression. Since P-gp and other ATP-binding cassette (ABC) transporters can limit the transport of drugs into the brain (Deeley et al., 2006) all CNS drugs needs to be tested with regard to interactions with these transport proteins.

Another point of clinical relevance for P-gp substrates is co-medication and the possibility of drug interactions. Some substances can increase the expression of P-gp and one example is the herbal antidepressant treatment St. John's Wort (Hennessy *et al.*, 2002). Such an interaction was reported for venlafaxine in an *in vitro* study (Ehret *et al.*, 2007). So either the medication itself or the co-medication possibly influences the treatment results via modulation of P-gp. This might explain why some patients do not reach sufficient drug concentrations in the brain and therefore fail to benefit from therapy. A number of substances have successfully been used to decrease (Ford and Hait, 1990) or increase the P-gp activity in cell cultures and animal experiments (Seelig, 1998). It can therefore be assumed that co-medications may counteract therapeutic effects in humans, explaining inter-individual differences in response to drugs that are substrate for P-gp.

In conclusion, the two major novel findings of the present study are that: (1) the S- and R-enantiomers of venlafaxine and its two main metabolites (Odm-venlafaxine and Ndm-venlafaxine) are substrates for P-gp, and (2) the P-gp affinity does not differ between the two enantiomers of venlafaxine or the metabolites. Accordingly, the present results confirm and extend previously obtained pharmacokinetic data on venlafaxine in the P-gp knockout mouse

model. Since several CNS-active drugs are given as racemic mixtures further research is warranted to predict the interaction of such drugs with P-gp at the BBB and thereby improve drug safety and therapeutics.

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Contributors

LK, JA, FB, FCK and CH were involved in the conception and design of the study. LK conducted the experiments and wrote the first draft of the manuscript. US assisted with the animal experiments and edited the manuscript. MJ and BC assisted with the drug analysis. FCK and CH supervised the project and edited the manuscript. All authors participated in the data interpretation and have approved the final manuscript.

Conflict of interest

None of the authors has conflicts of interest that interfere with the integrity of the content of the article.

References

- Ballabh, P., Braun, A., Nedergaard, M., 2004. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis* 16, 1-13.
- Beaulieu, E., Demeule, M., Ghitescu, L., Beliveau, R., 1997. P-glycoprotein is strongly expressed in the luminal membranes of the endothelium of blood vessels in the brain. *Biochem J* 326 (Pt 2), 539-544.
- Biegel, D., Spencer, D.D., Pachter, J.S., 1995. Isolation and culture of human brain microvessel endothelial cells for the study of blood-brain barrier properties in vitro. *Brain Res* 692, 183-189.
- Ciusani, E., Zullino, D.F., Eap, C.B., Brawand-Amey, M., Brocard, M., Baumann, P., 2004. Combination therapy with venlafaxine and carbamazepine in depressive patients not responding to venlafaxine: pharmacokinetic and clinical aspects. *J Psychopharmacol* 18, 559-566.
- Deeley, R.G., Westlake, C., Cole, S.P., 2006. Transmembrane transport of endo- and xenobiotics by mammalian ATP-binding cassette multidrug resistance proteins. *Physiol Rev* 86, 849-899.
- Devault, A., Gros, P., 1990. Two members of the mouse *mdr* gene family confer multidrug resistance with overlapping but distinct drug specificities. *Mol Cell Biol* 10, 1652-1663.
- Doran, A., Obach, R.S., Smith, B.J., Hosea, N.A., Becker, S., Callegari, E., Chen, C., Chen, X., Choo, E., Cianfrogna, J., Cox, L.M., Gibbs, J.P., Gibbs, M.A., Hatch, H., Hop, C.E., Kasman, I.N., Laperle, J., Liu, J., Liu, X., Logman, M., Maclin, D., Nedza, F.M., Nelson, F., Olson, E., Rahematpura, S., Raunig, D., Rogers, S., Schmidt, K., Spracklin, D.K., Szewc, M., Troutman, M., Tseng, E., Tu, M., Van Deusen, J.W., Venkatakrishnan, K., Walens, G., Wang, E.Q., Wong, D., Yasgar, A.S., Zhang, C., 2005. The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: evaluation using the MDR1A/1B knockout mouse model. *Drug Metab Dispos* 33, 165-174.
- Eap, C.B., Bertel-Laubscher, R., Zullino, D., Amey, M., Baumann, P., 2000. Marked increase of venlafaxine enantiomer concentrations as a consequence of metabolic interactions: a case report. *Pharmacopsychiatry* 33, 112-115.
- Eap, C.B., Lessard, E., Baumann, P., Brawand-Amey, M., Yessine, M.-A., O'Hara, G., Turgeon, J., 2003. Role of CYP2D6 in the stereoselective disposition of venlafaxine in humans. *Pharmacogenetics* 13, 39-47.
- Ehret, M.J., Levin, G.M., Narasimhan, M., Rathinavelu, A., 2007. Venlafaxine induces P-glycoprotein in human Caco-2 cells. *Hum Psychopharmacol* 22, 49-53.
- Ellingrod, V.L., Perry, P.J., 1994. Venlafaxine: a heterocyclic antidepressant. *Am J Hosp Pharm* 51, 3033-3046.
- Fogelman, S.M., Schmider, J., Venkatakrishnan, K., von Moltke, L.L., Harmatz, J.S., Shader, R.I., Greenblatt, D.J., 1999. O- and N-demethylation of venlafaxine in vitro by human liver

microsomes and by microsomes from cDNA-transfected cells: effect of metabolic inhibitors and SSRI antidepressants. *Neuropsychopharmacology* 20, 480-490.

Ford, J.M., Hait, W.N., 1990. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* 42, 155-199.

Fromm, M.F., 2000. P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. *Int J Clin Pharmacol Ther* 38, 69-74.

Fromm, M.F., 2002. Genetically determined differences in P-glycoprotein function: implications for disease risk. *Toxicology* 181-182, 299-303.

Gex-Fabry, M., Balant-Gorgia, A.E., Balant, L.P., Rudaz, S., Veuthey, J.L., Bertschy, G., 2004. Time course of clinical response to venlafaxine: relevance of plasma level and chirality. *Eur J Clin Pharmacol* 59, 883-891.

Gex-Fabry, M., Rudaz, S., Balant-Gorgia, A.E., Brachet, A., Veuthey, J.L., Balant, L.P., Bertschy, G., 2002. Steady-state concentration of venlafaxine enantiomers: model-based analysis of between-patient variability. *Eur J Clin Pharmacol* 58, 323-331.

Hennessy, M., Kelleher, D., Spiers, J.P., Barry, M., Kavanagh, P., Back, D., Mulcahy, F., Feely, J., 2002. St Johns wort increases expression of P-glycoprotein: implications for drug interactions. *Br J Clin Pharmacol* 53, 75-82.

Holliday, S.M., Benfield, P., 1995. Venlafaxine. A review of its pharmacology and therapeutic potential in depression. *Drugs* 49, 280-294.

Jaquenoud Sirot, E., Knezevic, B., Morena, G.P., Harenberg, S., Oneda, B., Crettol, S., Ansermot, N., Baumann, P., Eap, C.B., 2009. ABCB1 and cytochrome P450 polymorphisms: clinical pharmacogenetics of clozapine. *J Clin Psychopharmacol* 29, 319-326.

Kirschbaum, K.M., Henken, S., Hiemke, C., Schmitt, U., 2008. Pharmacodynamic consequences of P-glycoprotein-dependent pharmacokinetics of risperidone and haloperidol in mice. *Behav Brain Res* 188, 298-303.

Lagas, J.S., Vlaming, M.L., Schinkel, A.H., 2009. Pharmacokinetic assessment of multiple ATP-binding cassette transporters: the power of combination knockout mice. *Mol Interv* 9, 136-145.

Linnet, K., Ejning, T.B., 2008. A review on the impact of P-glycoprotein on the penetration of drugs into the brain. Focus on psychotropic drugs. *Eur Neuropsychopharmacol* 18, 157-169.

Luurtsema, G., Molthoff, C.F., Windhorst, A.D., Smit, J.W., Keizer, H., Boellaard, R., Lammertsma, A.A., Franssen, E.J., 2003. (R)- and (S)-[11C]verapamil as PET-tracers for measuring P-glycoprotein function: in vitro and in vivo evaluation. *Nucl Med Biol* 30, 747-751.

Miura, M., Uno, T., Tateishi, T., Suzuki, T., 2007. Pharmacokinetics of fexofenadine enantiomers in healthy subjects. *Chirality* 19, 223-227.

- Muth, E.A., Haskins, J.T., Moyer, J.A., Husbands, G.E., Nielsen, S.T., Sigg, E.B., 1986. Antidepressant biochemical profile of the novel bicyclic compound Wy-45,030, an ethyl cyclohexanol derivative. *Biochem Pharmacol* 35, 4493-4497.
- Muth, E.A., Haskins, J.T., Moyer, J.A., Andree, T.H., Husbands, G.E.M., 1991. Biochemical, neuropsychological, and behavioral effects of Wy-45,233 and other identified metabolites of the antidepressant venlafaxine. *Drug Dev Res* 23, 191-199.
- Oganesian, A., Shilling, A.D., Young-Sciame, R., Tran, J., Watanyar, A., Azam, F., Kao, J., Leung, L., 2009. Desvenlafaxine and venlafaxine exert minimal in vitro inhibition of human cytochrome p450 and p-glycoprotein activities. *Psychopharmacol Bull* 42, 47-63.
- Otton, S.V., Ball, S.E., Cheung, S.W., Inaba, T., Rudolph, R.L., Sellers, E.M., 1996. Venlafaxine oxidation in vitro is catalysed by CYP2D6. *Br J Clin Pharmacol* 41, 149-156.
- Pirraglia, P.A., Stafford, R.S., Singer, D.E., 2003. Trends in Prescribing of Selective Serotonin Reuptake Inhibitors and Other Newer Antidepressant Agents in Adult Primary Care. *Prim Care Companion J Clin Psychiatry* 5, 153-157.
- Rochat, B., Baumann, P., Audus, K.L., 1999. Transport mechanisms for the antidepressant citalopram in brain microvessel endothelium. *Brain Res* 831, 229-236.
- Sandstrom, R., Karlsson, A., Lennernas, H., 1998. The absence of stereoselective P-glycoprotein-mediated transport of R/S-verapamil across the rat jejunum. *J Pharm Pharmacol* 50, 729-735.
- Schinkel, A.H., Mayer, U., Wagenaar, E., Mol, C.A., van Deemter, L., Smit, J.J., van der Valk, M.A., Voordouw, A.C., Spits, H., van Tellingen, O., Zijlmans, J.M., Fibbe, W.E., Borst, P., 1997. Normal viability and altered pharmacokinetics in mice lacking *mdr1*-type (drug-transporting) P-glycoproteins. *Proc Natl Acad Sci U S A* 94, 4028-4033.
- Schinkel, A.H., Smit, J.J., van Tellingen, O., Beijnen, J.H., Wagenaar, E., van Deemter, L., Mol, C.A., van der Valk, M.A., Robanus-Maandag, E.C., te Riele, H.P., et al., 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.
- Seelig, A., 1998. A general pattern for substrate recognition by P-glycoprotein. *Eur J Biochem* 251, 252-261.
- Smith, S.W., 2009. Chiral toxicology: it's the same thing...only different. *Toxicol Sci* 110, 4-30.
- Sonnenberg, C.M., Deeg, D.J., Comijs, H.C., van Tilburg, W., Beekman, A.T., 2008. Trends in antidepressant use in the older population: results from the LASA-study over a period of 10 years. *J Affect Disord* 111, 299-305.
- Tsuji, A., Terasaki, T., Takabatake, Y., Tenda, Y., Tamai, I., Yamashita, T., Moritani, S., Tsuruo, T., Yamashita, J., 1992. P-glycoprotein as the drug efflux pump in primary cultured bovine brain capillary endothelial cells. *Life Sci* 51, 1427-1437.

Uhr, M., Grauer, M.T., 2003. abcb1ab P-glycoprotein is involved in the uptake of citalopram and trimipramine into the brain of mice. *J Psychiatr Res* 37, 179-185.

Uhr, M., Grauer, M.T., Holsboer, F., 2003. Differential enhancement of antidepressant penetration into the brain in mice with abcb1ab (mdr1ab) P-glycoprotein gene disruption. *Biol Psychiatry* 54, 840-846.

Uhr, M., Tontsch, A., Namendorf, C., Ripke, S., Lucae, S., Ising, M., Dose, T., Ebinger, M., Rosenhagen, M., Kohli, M., Kloiber, S., Salyakina, D., Bettecken, T., Specht, M., Putz, B., Binder, E.B., Muller-Myhsok, B., Holsboer, F., 2008. Polymorphisms in the drug transporter gene ABCB1 predict antidepressant treatment response in depression. *Neuron* 57, 203-209.

Wang, C.P., Howell, S.R., Scatina, J., Sisenwine, S.F., 1992. The disposition of venlafaxine enantiomers in dogs, rats, and humans receiving venlafaxine. *Chirality* 4, 84-90.

Wang, J.S., Ruan, Y., Taylor, R.M., Donovan, J.L., Markowitz, J.S., DeVane, C.L., 2004. Brain penetration of methadone (R)- and (S)-enantiomers is greatly increased by P-glycoprotein deficiency in the blood-brain barrier of Abcb1a gene knockout mice. *Psychopharmacology (Berl)* 173, 132-138.

Wikell, C., Eap, C.B., Josefsson, M., Apelqvist, G., Ahlner, J., Baumann, P., Bengtsson, F., 2002. Disposition of venlafaxine enantiomers in rats with hepatic encephalopathy after chronic drug treatment. *Chirality* 14, 347-350.

Table 1. Total (S+R) concentrations of venlafaxine, O-desmethylenlafaxine (Odm-venlafaxine) and N-desmethylenlafaxine (Ndm-venlafaxine) in *abcb1ab* (-/-) and *abcb1ab* (+/+) mice at four different time points after receiving 10 mg/kg racemic venlafaxine (n=10 at each time point).

Substance	Time	Serum				Brain			
		<i>abcb1ab</i> (-/-) (nmol/L)		<i>abcb1ab</i> (+/+) (nmol/L)		<i>abcb1ab</i> (-/-) (nmol/L)		<i>abcb1ab</i> (+/+) (nmol/L)	
		mean ± SEM	mean ± SEM	Ratio	p value	mean ± SEM	mean ± SEM	Ratio	p value
Venlafaxine	1h	983 ± 163	918 ± 147	1.07	<i>ns</i>	8797 ± 1164	3731 ± 406	2.36	<0.001
	3h	113 ± 29.3	61.7 ± 6.1	1.83	<i>ns</i>	837 ± 218	266 ± 20.2	3.15	<0.05
	6h	10.2 ± 0.97	5.4 ± 0.77	1.89	<0.01	55.6 ± 4.2	20.0 ± 2.7	2.78	<0.001
	9h	2.1 ± 0.17	2.3 ± 0.37	0.91	<i>ns</i>	10.0 ± 1.1	9.1 ± 1.1	1.10	<i>ns</i>
Odm-Venlafaxine	1h	108 ± 13.6	97.5 ± 8.4	1.11	<i>ns</i>	421 ± 28.3	169 ± 9.4	2.49	<0.001
	3h	24.5 ± 4.6	14.7 ± 1.2	1.67	<i>ns</i>	295 ± 30.7	73.5 ± 4.2	4.01	<0.001
	6h	5.3 ± 0.47	3.3 ± 0.31	1.61	<0.05	126 ± 7.5	20.3 ± 1.2	6.21	<0.001
	9h	3.1 ± 0.62	1.5 ± 0.17	2.07	<0.05	49.3 ± 4.4	7.9 ± 0.28	6.24	<0.001
Ndm-Venlafaxine	1h	383 ± 51.2	414 ± 54.1	0.93	<i>ns</i>	1019 ± 95.9	327 ± 18.6	3.12	<0.001
	3h	77.3 ± 14.6	56.9 ± 8.4	1.36	<i>ns</i>	894 ± 63.4	190 ± 20.2	4.71	<0.001
	6h	7.5 ± 0.69	4.4 ± 0.50	1.70	<0.01	460 ± 20.6	39.8 ± 4.3	11.56	<0.001
	9h	1.5 ± 0.14	1.0 ± 0.07	1.50	<0.05	179 ± 13.9	9.3 ± 0.56	19.25	<0.001

ns= not significant

P-values < 0.05 indicate significant differences between *abcb1ab* (-/-) and *abcb1ab* (+/+) (unpaired two-tailed Student's *t*-test).

Table 2. Enantiomeric S/R concentration ratios of venlafaxine, Odm-venlafaxine and Ndm-venlafaxine, in *abcb1ab* knockout mice and wildtype mice at four different time points after single injection of 10 mg/kg racemic venlafaxine (n=10 at each time point).

Substance	Time	Serum			Brain		
		abcb1 ab (-/-)	abcb1ab (+/+)	p value	abcb1 ab (-/-)	abcb1ab (+/+)	p value
		S/R ratio	S/R ratio		S/R ratio	S/R ratio	
Venlafaxine	1h	1.16	1.12	<i>ns</i>	1.12	1.42	< 0.001
	3h	1.38	1.24	<i>ns</i>	1.28	1.52	< 0.01
	6h	1.42	1.31	<i>ns</i>	1.27	1.44	< 0,05
	9h	1.18	1.14	<i>ns</i>	1.13	1.23	<i>ns</i>
Odm-Venlafaxine	1h	1.90	1.75	< 0,05	1.68	1.68	<i>ns</i>
	3h	1.94	1.69	< 0.01	1.77	1.76	<i>ns</i>
	6h	1.93	1.82	<i>ns</i>	1.93	2.11	<i>ns</i>
	9h	1.60	1.40	<i>ns</i>	2.13	1.42	< 0.001
Ndm-Venlafaxine	1h	2.65	2.36	<i>ns</i>	1.94	2.32	< 0.01
	3h	3.47	2.77	<i>ns</i>	2.02	2.12	<i>ns</i>
	6h	2.74	2.33	<i>ns</i>	2.09	2.13	<i>ns</i>
	9h	1.93	1.71	<i>ns</i>	2.04	1.91	<i>ns</i>

ns= not significant

P-values < 0.05 indicate significant differences between *abcb1ab* (-/-) and *abcb1ab* (+/+) (unpaired two-tailed Student's *t*-test).

Figure legends

Figure 1. Enantiomeric (S and R) concentrations of venlafaxine, O-desmethylvenlafaxine and N-desmethylvenlafaxine in serum and brain after single i.p. injection of racemic venlafaxine (10 mg/kg) to *abcb1ab* (-/-) and *abcb1ab* (+/+) mice 1h (*top panel*), 3h (*middle panel*) and 6h (*bottom panel*) after administration of drug. Values are means $\pm$ SEM (n = 10). *p<0.05, **p<0.01, ***p<0.001

Figure 2. Brain/serum ratios of venlafaxine, O-desmethylvenlafaxine and N-desmethylvenlafaxine in *abcb1ab* (-/-) and *abcb1ab* (+/+) mice after single i.p. injection of racemic venlafaxine (10 mg/kg). Values are means $\pm$ SEM (n = 10). *p<0.05, **p<0.01, ***p<0.001

Figure 1

Figure 2