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Effects on enantiomeric drug disposition and open-field behavior after chronic treatment with venlafaxine in the P-glycoprotein knockout mice model

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Abstract

Rationale P-glycoprotein (P-gp) plays an important role in the efflux of drugs from the brain back into the blood stream and can influence the pharmacokinetics and –dynamics of drug molecules. To our knowledge, no studies have reported pharmacodynamic effects of any antidepressant drug in the P-gp knockout mice model.

Objective The aim of this study was to investigate the enantiomeric venlafaxine and metabolite concentrations in serum and brain of *abcb1ab (-/-)* mice compared to wildtype mice upon chronic dosing, and to assess the effect of venlafaxine treatment on open-field behavior.

Methods P-gp knockout and wildtype mice received two daily intraperitoneal injections of venlafaxine (10 mg/kg) over ten consecutive days. Locomotor and rearing activities were assessed on day 7 and 9. After ten days, drug and metabolite concentrations in brain and serum were determined using an enantioselective LC/MS/MS method.

Results The brain concentrations of venlafaxine and its three demethylated metabolites were 2-4 times higher in *abcb1ab (-/-)* mice compared to *abcb1ab (+/+)* mice. The behavioral results indicated an impact on exploration-related behaviors in the open-field as center activity was increased and rears were decreased by venlafaxine treatment.

Conclusion Our results show that P-gp at the blood-brain barrier plays an important role in limiting brain entry of the enantiomers of venlafaxine and its metabolites after chronic dosing. Taken together, the present pharmacokinetic and pharmacodynamic findings offer the possibility that the expression of P-gp in patients may be a contributing factor for limited treatment response.

Keywords abcb1ab, Blood-brain barrier, Knockout mice, P-glycoprotein, Pharmacodynamic, Pharmacokinetic, Venlafaxine

Introduction

Depression is a common and significant public health problem, and the leading cause of suicide worldwide (Kessler et al. 2009; Moussavi et al. 2007). Pharmacological treatment is one of the cornerstones in clinical practice, but however, it is well known that the result of antidepressant therapy can vary within a population. One factor contributing to this might be the ability of the drug to cross the blood-brain barrier (BBB). Two important functions of the BBB are protection of the brain against potentially toxic substances and maintenance of a constant internal environment, which is of primary importance for optimal neuronal functioning of the brain. The BBB expresses transport proteins which actively transport their substrates out of the brain back into the blood circulation (Ballabh et al. 2004). One of the most studied transport proteins is P-glycoprotein (P-gp) (Cordon-Cardo et al. 1989; Thiebaut et al. 1987), which belongs to the highly conserved superfamily of ATP-binding cassette (ABC) transporter proteins (Ambudkar et al. 1999). The importance of P-gp in the transport of drugs over the BBB has been studied using *abcb1ab* knockout mice (Schinkel et al. 1997; Schinkel et al. 1994), thus providing a unique and valuable pharmacological tool.

A well-known problem in modern psychopharmacology is the existence of chiral drugs composed of enantiomers which may differ in their pharmacokinetic and pharmacodynamic properties (Baker et al. 2002; Baumann et al. 2002; Howland 2009). One example of such a drug is venlafaxine, which inhibits serotonin more than noradrenaline reuptake and slightly inhibits dopamine reuptake (Muth et al. 1986). Venlafaxine is used for the treatment of major depressive disorder, generalized anxiety disorder, social anxiety disorder, and panic disorder (Stahl et al. 2005). Venlafaxine exists as a racemic mixture of the R-(–)- and S-(+)-enantiomeric forms in equal amounts, both of which inhibits monoamine reuptake (Muth et al. 1986). However, the R-enantiomer is a potent inhibitor of both serotonin and noradrenaline reuptake, whereas the S-enantiomer is more selective in inhibiting serotonin reuptake

(Holliday et al. 1995; Muth et al. 1986). Venlafaxine is mainly metabolized by the cytochrome P450 (CYP) enzyme CYP2D6 to its major active metabolite O-desmethylvenlafaxine (Odm-venlafaxine) and to its two minor metabolites, N-desmethylvenlafaxine (Ndm-venlafaxine) and N,O-didesmethylvenlafaxine (Ddm-venlafaxine) (Muth et al. 1991; Muth et al. 1986).

Several pharmacokinetic studies have been published using the *abcb1ab* knockout mouse model and P-gp has been shown to significantly limit brain entry of a wide variety of drugs including venlafaxine (Doran et al. 2005; Kirschbaum et al. 2010; Uhr et al. 2000; Uhr and Grauer 2003; Uhr et al. 2003; Uhr et al. 2008). Using this animal model, we have recently shown that P-gp decreases the brain penetration of the S- and R-enantiomers of venlafaxine and its metabolites after an acute drug challenge (Karlsson et al. 2010). However, no enantiomeric pharmacokinetic data are available following chronic drug administration. Further, to our knowledge, no studies have reported pharmacodynamic effects of any antidepressant drug to P-gp deficient mice and its wildtype controls. The open-field test is the method of choice for analysing an animal's spontaneous behavior in response to a novel environment (Avgustinovich et al. 2000; Gershenfeld et al. 1997). Following a drug challenge this test in rodents gives hints for main clinical effects (Apelqvist et al. 1999; Kennett et al. 1987; Kugelberg et al. 2002; Kugelberg et al. 2005; Kugelberg et al. 2006).

To obtain results that are more closely related to the clinical situation we decided to follow up our previous acute study (Karlsson et al. 2010) by chronic treatment with venlafaxine in the P-gp knockout mouse model. The aims of the study were to investigate: (1) the distribution of the enantiomers of venlafaxine and its three major metabolites in serum and brain after ten days of drug administration; and (2) the effect of chronic drug treatment on open-field behavior.

Materials and methods

Animals

Male *abcb1ab (-/-)* mice (mean weight 35 g) on a FVB/N background (Taconic, Germantown, NY) and male FVB/N wild type mice (*abcb1ab (+/+)*) (mean weight 31 g) were housed in groups of five 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. All experiments were conducted in accordance to the European guide for care and use of laboratory animals and approved by local authorities.

Drugs and chemicals

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

Experimental design

For the pharmacokinetics, venlafaxine was administered to *abcb1ab (-/-)* knockout (*n*=10) and *abcb1ab (+/+)* FVB/N wild type (*n*=10) mice by daily i.p. injections during a brief anesthesia with isoflurane (Forene®, Abbott GmbH & Co. KG, Wiesbaden, Germany). The concentration used was 10 mg/kg body weight and the volume injected twice daily (8:00 am and 8:00 pm) was 1 mL giving a total dose of 20 mg/kg/day. The choice of dosage regimen was based on previous pharmacokinetic (Uhr et al. 2003; Karlsson et al. 2010) and pharmacodynamic (Kumar et al. 2010; Takeuchi et al. 2010) studies in which doses within the range 5-20 mg/kg were used. Ten days following drug administration, the knockout and

wildtype mice were anesthetized with isoflurane and decapitated 1 hour after the last injection. Serum and brain samples were then collected and frozen at -70°C until further analysis of drug and metabolite concentrations (for details, see below).

For the pharmacodynamics, the number of animals was extended. Venlafaxine was administered to *abcb1ab (-/-)* knockout (*n*=16) and *abcb1ab (+/+)* FVB/N wild type (*n*=15) mice by daily i.p. injections as described above. Control mice of each genotype (knockout *n*=14 and wildtype *n*=10) were injected with saline instead of venlafaxine. Behavioral testing was performed seven and nine days after the start of the drug administration (for details, see below).

Behavioral tests

Exploratory locomotion/activity in mice was assessed in an open-field paradigm. The test arena consisted of dark-gray plastic and measured 60 cm x 60 cm x 35 cm. The arena was virtually divided in several parts, corners with 15 cm x 15 cm, corridors near the wall with 8 cm width and a center-square with again 15 cm x 15 cm. That allows, in addition, division of the floor of the arena into two similar large areas a peripheral (corners and walled part) and central (center area and rest). The mouse was allowed to explore the arena for 10 min. Track path was subsequently analyzed by the automated system for the following parameters: distance travelled (cm), time spent in the various parts of the arena and the number of rears. Behavioral testing was performed 30 min after drug injection on day 7 and 9. All behavioral tests were conducted between 9:00 am and 2:00 pm. The hardware consisted of an IBM-type computer combined with a video digitizer and a CCD video camera (Panasonic, CCTV Camera WVBP330/GE, Sushou, China). The software used was EthoVision® release 3.1 (Noldus Information Technology, Utrecht, The Netherlands).

Drug analysis

The concentrations of the S- and R-enantiomers of venlafaxine and its metabolites in serum (nmol/L) and brain homogenate supernatant (pmol/g ≈ nmol/L) were determined by using a liquid chromatography tandem mass spectrometry (LC/MS/MS) method as described previously (Karlsson et al. 2010; Kingbäck et al. 2010). Briefly, the whole brain samples (mean weight 400 mg) were homogenized with a sonifier (Sonics VibraCell VC 130; Chemical Instruments AB) in 2 mL Milli-Q water and centrifuged at 2000g for 20 min. Venlafaxine, Odm-venlafaxine, Ndm-venlafaxine and Ddm-venlafaxine were extracted from 0.2 mL serum and 0.2 mL brain homogenate supernatant with solid-phase extraction on Isolute C8 columns 100 mg (International Sorbent Technology Ltd). After elution and evaporation, the samples were redissolved in 50 µL of the mobile phase (tetrahydrofuran:ammonium acetate, 10 mM, pH 6; 10:90 v/v) before the analysis. The LC/MS/MS system consisted of an Acquity liquid chromatography system (Waters) and an API 4000 tandem quadrupole instrument equipped with an electrospray interface (TURBO V™ source, TurbolonSpray® probe) operating in positive ion mode (Applied Biosystem/MSD Sciex). Chromatographic separation was performed on a Chirobiotic-V column (5 µm particle size, 250 x 2.1 mm; Astec/Supelco) protected with a 5 µm in-line filter (VICI AB Int.) at a mobile phase flow rate of 0.2 mL/min. The column was kept at 10°C using a Jones Chromatography Model 7955 column chiller/heater. Multiple reaction monitoring (MRM) of the most abundant transitions originating from product ions of the protonated molecular ions was used for quantification. The data were processed using Analyst 1.4.2 software (Applied Biosystem/MSD Sciex). The limit of quantification was 0.5 nmol/L for the enantiomers of venlafaxine and Odm-venlafaxine, and 0.25 nmol/L for the enantiomers of Ndm-venlafaxine and Ddm-venlafaxine.

Extraction recoveries and matrix effects for the brain samples were investigated in the same way as previously described for plasma (Kingbäck et al. 2010). The extraction recovery in brain homogenate supernatant was determined by comparing extracted spiked blank samples with unextracted reference samples prepared at the same concentrations. Matrix effects were evaluated by comparing the concentrations found of known amounts of working standards with those measured in control brain homogenate supernatant spiked with the same amount of analytes before or after extraction. Brain samples at two different concentration levels (100 and 1000 nmol/L for the enantiomers of venlafaxine and Odm-venlafaxine; 50 and 500 nmol/L for Ndm-venlafaxine and Ddm-venlafaxine) were analyzed. The mean extraction recoveries for the enantiomers of venlafaxine and its metabolites ranged between 75-91% and 74-93%, respectively. The matrix effect data for venlafaxine and metabolites were between 99-105% and 80-88%, respectively, indicating no severe matrix effects.

Statistical analysis

Data are expressed as means ± the standard error of the means (SEM). Pharmacokinetic data were analyzed by a two-tailed Student's *t*-test for unpaired observations using StatView® for Windows Version 5.0 (SAS® Institute Inc., Cary, NC; USA). Behavioral data were analyzed by analysis of variance (ANOVA) followed by a post-hoc *t*-test using SPSS® for Windows Version 12.0 (SPSS Inc., Chicago, IL, USA). For all comparisons p-values <0.05 were considered statistically significant.

Results

Concentrations of the enantiomers of venlafaxine and metabolites

The total (S+R) concentrations, the concentrations of the S- and R-enantiomers and the S/R concentration ratios of venlafaxine and its metabolites are displayed in Table 1. In the brain, the S- and R-enantiomeric concentrations of venlafaxine were found to be 1.6 and 2.4 times higher in the *abcb1ab* knockout mice as compared to the wild type mice, respectively ($p<0.0001$). For the metabolites, the S- and R-enantiomeric concentrations were 2.1 and 2.1 times higher for Odm-venlafaxine, 3.2 and 4.5 times higher for Ndm-venlafaxine and 2.0 and 2.1 times higher for Ddm-venlafaxine in the knockout mice than in the wild type mice, respectively ($p<0.0001$). No significant differences were observed in the serum S- and R-enantiomeric concentrations of the knockout mice as compared to the wild type mice, with one exception for R-venlafaxine ($p=0.02$).

For venlafaxine and Ndm-venlafaxine, the enantiomeric S/R concentration ratios in brain were 1.02 and 1.35 in P-gp deficient mice, respectively, as compared to 1.40 and 1.87 in wildtype mice and these differences between the groups were statistically significant ($p<0.0001$) (Fig. 1). However, the S/R ratios in brain for Odm-venlafaxine and Ddm-venlafaxine did not differ between the *abcb1ab* knockout mice and wild type mice (1.60 vs. 1.64 for Odm-venlafaxine and 1.99 vs. 2.08 for Ddm-venlafaxine). The serum S/R ratios differed statistically between the groups (p-values ranging from <0.05 to <0.001), even though no differences were found in the S- and R-concentrations of parent drug and metabolites with one exception (R-venlafaxine $p<0.05$). Further, when comparing the knockout/wildtype ratios for the S- to R-enantiomers of venlafaxine and metabolites in brain, no major differences between the groups were found. 1.8 for S-venlafaxine compared to 2.4

for R-venlafaxine, 2.1 for S-Odm-venlafaxine compared to 2.1 for R-Odm-venlafaxine and 3.2 for S-Ndm-venlafaxine compared to 4.5 for R-Ndm-venlafaxine.

In mice lacking P-gp, the brain/serum concentration ratio of venlafaxine was significantly higher compared to wild type mice ($p<0.0001$) (Fig. 2). The brain/serum concentration ratios of Odm-venlafaxine, Ndm-venlafaxine and Ddm-venlafaxine were found to be 1.7-3.2 times higher in the *abcb1ab* knockout mice as compared to the wild type mice ($p<0.0001$, $p<0.0001$ and $p<0.0005$) (Fig. 2).

The metabolite/parent drug (M/P) ratios for Odm-venlafaxine/venlafaxine, Ndm-venlafaxine/venlafaxine and Ddm-venlafaxine/venlafaxine in serum were similar between the two groups (0.15, 1.87 and 0.02 in knockout mice vs. 0.16, 1.91 and 0.02 in wildtype mice, respectively). In brain, the M/P ratios for Odm-venlafaxine/venlafaxine and Ddm-venlafaxine/venlafaxine were 0.06 and 0.001 in *abcb1ab* knockout and wildtype mice. The brain M/P ratios for Ndm-venlafaxine/venlafaxine were 0.7 in knockout mice and 0.4 in wildtype mice ($p<0.0001$).

Open-field behavior

Distance travelled (locomotion) in the open-field arena was analyzed as a general measure of activity whereas time spent in various zones and number of rears was used as a measure of exploratory activity. There were no differences in general activity (total locomotion) between the groups after seven days ($F_{(3;51)}=2.369$; $p=0.08$) of chronic drug treatment (Fig. 3). Nine days after treatment, and after having been in the test before, activity appeared significantly different ($F_{(3;51)}=4.394$; $p\leq0.01$). Post-hoc analysis indicated that this decrease in activity was based on a significant effect ($p=0.011$) of venlafaxine treatment in wildtype mice. There were no treatment differences in *abcb1ab* knockout mice ($p=0.074$) (Fig. 3).

Investigating exploration by analysis of the time spent in the periphery or central area showed significant treatment effects after seven and nine days of drug administration ($F_{(3;51)}$=2.716; $p \leq 0.05$ and $F_{(3;51)}$=4.111; $p \leq 0.01$, respectively). Post-hoc comparisons showed that *abcb1ab* knockout mice treated for seven days with venlafaxine spent significantly more time in the central area compared to similar treated wildtype (p=0.031) and untreated *abcb1ab* knockout mice (p=0.022) (Fig. 4). After nine days of chronic treatment post-hoc comparison revealed a significant difference to untreated *abcb1ab* knockout mice only (p=0.002) (Fig. 4) indicating that treatment effects in wildtype animals need longer time to emerge. Locomotor activity within the various zones displayed only a significant effect in the periphery nine days after treatment ($F_{(3;51)}$=5.043; $p \leq 0.01$ seven days: $F_{(3;51)}$=1.871; p=0.146) with post-hoc comparison showing a clear venlafaxine effect in both genotypes (wildtype p=0.014, *abcb1ab* knockout p=0.025) (Fig. 3). Analysis of rears indicated no significant differences in the central area after seven and nine days of chronic drug administration ($F_{(3;51)}$=1.123; p=0.384; $F_{(3;51)}$=1.413; p=0.250), whereas in the periphery there was a significant effect after nine days of treatment ($F_{(3;51)}$=3.773; $p \leq 0.05$) while after seven days ANOVA did not show any significance ($F_{(3;51)}$=2.415; p=0.077). However, post-hoc comparison indicated that after seven days *abcb1ab* knockout mice treated with venlafaxine showed less rears compared to untreated (p=0.034) and wildtype mice (p=0.020) receiving venlafaxine. After nine days, venlafaxine in both genotypes reduced the number of rears significantly (wildtype p=0.030; *abcb1ab* knockout p=0.017) again indicating a faster onset of treatment effect in *abcb1ab* knockout mice (Fig. 4).

Discussion

The present study was designed to combine pharmacokinetic and pharmacodynamic investigations in the P-gp knockout mouse model after chronic treatment with venlafaxine. To

our knowledge, this approach has not previously been applied after administration of any antidepressant drug. We report that the brain concentrations of venlafaxine and its three metabolites were 2-4 times higher in P-gp deficient mice compared to wildtype controls. Despite these differences the enantiomeric drug and metabolite dispositions in serum and brain were similar between the two genotypes. The open-field test indicated an impact on exploration-related behaviors in knockout mice as center activity was increased and rears were decreased by chronic venlafaxine treatment.

P-gp is responsible for transporting a substrate back into the blood thereby inhibiting the transport of certain substances, including drugs, to enter the brain. This means that P-gp may prevent certain drugs from reaching the central nervous system (CNS) in therapeutic doses. Studies have shown that the activity of the gene coding for P-gp, *ABCB1*, varies between individuals, which means that drug concentrations in the brain is dependent on P-gp levels (Kroetz et al. 2003). A high level of gene activity may be the reason why a patient does not reach sufficiently high concentrations in the brain and therefore may not get the correct effect of the treatment. Several single-nucleotide polymorphisms (SNPs) identified in the human *ABCB1* gene have been associated with altered P-gp expression and phenotype (Hoffmeyer et al. 2000; Kim et al. 2001; Tanabe et al. 2001) and some of the polymorphisms have been connected with antidepressant response (Gex-Fabry et al. 2008; Kato et al. 2008; Nikisch et al. 2008; Uhr et al. 2008). Accordingly, it is of clinical value to detect whether a particular drug given to treat a CNS disease is a substrate for P-gp.

Whereas P-gp is encoded by a single gene in humans (*ABCB1*), mice have two homologues, the *abcb1a* and *abcb1b* genes (Devault et al. 1990). To identify which drugs that are substrates of P-gp, mice lacking both the *abcb1a* and *abcb1b* genes have been used. This animal model shows that antidepressants like citalopram, doxepin, venlafaxine and paroxetine are substrates of P-gp at the BBB (Karlsson et al. 2010; Lagas et al. 2009; Uhr and Grauer

2003; Uhr et al. 2003). In the present study, venlafaxine was administered for ten days to investigate the time-dependent interaction between the drug and P-gp. Using a stereospecific LC/MS/MS method we analyzed the enantiomeric concentrations and showed that the separate enantiomers of the parent compound and its three metabolites are substrates of P-gp. This was evidenced by 2-4 times higher brain concentrations of venlafaxine, Odm-venlafaxine, Ndm-venlafaxine and Ddm-venlafaxine in the *abcb1ab (-/-)* mice as compared to the *abcb1ab (+/+)* mice. In a recent study performed by us (Karlsson et al. 2010), where *abcb1ab* knockout mice received a single i.p. injection of venlafaxine, we measured up to 2-fold higher brain concentrations of venlafaxine, whereas the brain concentrations of Odm-venlafaxine and Ndm-venlafaxine were 2-3 times higher than in wildtype mice 1 h post dose. In a chronic study by Uhr and co-workers, 1.7- and 4.1-fold higher brain concentrations of venlafaxine and Odm-venlafaxine were observed in *abcb1ab* knockout mice than in wildtype mice (Uhr et al. 2008).

The serum and brain concentrations of venlafaxine and Odm-venlafaxine were found to be similar following both acute (Karlsson et al. 2010) and chronic drug administration to *abcb1ab* knockout and wildtype mice. In the literature, little is known about the pharmacological profiles of Odm-venlafaxine and the two other demethylated metabolites and, to our knowledge, no data are available describing the effects of the separate enantiomers. Desvenlafaxine is a newly marketed antidepressant of the serotonin-noradrenaline reuptake inhibitor (SNRI) class. Desvenlafaxine is a synthetic form of the isolated major active metabolite (Odm-venlafaxine) of venlafaxine. According to a product monograph, desvenlafaxine is not a substrate or an inhibitor for the P-gp transporter in vitro (WYETH 2009). Further, the pharmacokinetics of desvenlafaxine is unlikely to be affected by drugs that inhibit the P-gp transporter and desvenlafaxine is not likely to affect the pharmacokinetics of drugs that are substrates of the P-gp transporter (WYETH 2009).

However, in the product monograph it is not stated in which type of cells these data were obtained. Nevertheless, these findings are not in line with our in vivo results, where major differences in brain concentrations of Odm-venlafaxine between the *abcb1ab* knockout mice and the wildtype mice were observed. However, other available data from the literature is also inconsistent. For example, studies in Caco-2 cells have given contradicting results for venlafaxine (Ehret et al. 2007; Oganesian et al. 2009). The antidepressant drug citalopram is another example where different results have been reported in vitro (Rochat et al. 1999) and in vivo (Uhr and Grauer 2003; Doran et al. 2005). To study Odm-venlafaxine (desvenlafaxine) and Ndm-venlafaxine separately in our mice model would be of high interest to further elucidate this important issue.

The given dose of venlafaxine (20 mg/kg/day) in the present study has previously been estimated to approach the human therapeutic serum level (de Klerk et al. 2009). The mean serum levels of venlafaxine were 1277 and 1083 nmol/L in knockout and wildtype mice, respectively, which are levels seen in the upper range in therapeutic drug monitoring (TDM) in humans (Reis et al. 2002). The metabolite/parent drug (M/P) ratios in serum for Odm-venlafaxine/venlafaxine, Ndm-venlafaxine/venlafaxine and Ddm-venlafaxine/venlafaxine were 0.16, 1.9 and 0.02 in wildtype mice. Comparing these ratios to patients, Reis and co-workers reported mean serum ratios of 5.5, 0.6 and 2.1, respectively (Reis et al. 2002). However, the inter-individual variability showed a wide range (min-max: 0.01-35, 0.01-7.8 and 0.08-30, respectively). In another TDM study, an Odm-venlafaxine/venlafaxine ratio of 2.5 was reported (Reis et al. 2009). Compared to humans, the mice in the present study displayed higher concentrations of Ndm-venlafaxine in relation to Odm-venlafaxine (Odm-venlafaxine/Ndm-venlafaxine ratio: 0.08 in mice and 2.5 in humans) (Reis et al. 2002). Taken together, there seems to be species differences in the metabolic pattern, shown by M/P ratios, that needs to be considered when results from different studies are compared. One also has to

keep in mind how the drug was administered and the time of sampling in relation to the time when the drug was given. In TDM trough levels are utilized which requires blood sampling within a drug specific time interval after dosing and steady state.

In the present study we separately investigated the disposition of the S- and R-enantiomers of venlafaxine and metabolites in serum and brain. The *abcb1ab* knockout mice displayed somewhat lower S/R concentration ratios of venlafaxine and Ndm-venlafaxine as compared to the wildtype mice. Also for Odm-venlafaxine a minor difference in S/R ratios was observed between the groups in serum but not in the brain. However, when comparing the knockout/wildtype ratios for the S- to R-enantiomers of venlafaxine and metabolites in brain no major differences between the groups were found. Hence, no major differences in stereoselective disposition of venlafaxine, Odm-venlafaxine, Ndm-venlafaxine and Ddm-venlafaxine were found in the *abcb1ab* knockout mice model. For S/R-verapamil, an absence of stereoselective P-gp mediated transport has been reported (Luurtsema et al. 2003; Sandstrom et al. 1998). It has to be taken into account that we only tested one dose. P-gp might be a saturable transporter, and lower or higher doses of the drug could lead to results that would differ between the groups. P-gp exports antidepressants at different rates, for instance when comparing the brain penetration for *abcb1ab (-/-)* to wildtype mice, the ratio for doxepin is low (1.2) (Uhr et al. 2003) and for venlafaxine higher (2.0) (present study). Compared to stronger P-gp substrates like digoxine (ratio 19) (Mayer et al. 1997), the ratios of the antidepressants are quite low but might still be of clinical relevance (Uhr et al. 2008).

To our knowledge, pharmacodynamic investigations of venlafaxine or any other antidepressant have not previously been performed in the *abcb1ab* knockout mouse model. In the literature, only limited data are available addressing the influence of P-gp on the pharmacodynamics using P-gp knockout mice (Kalvass et al. 2007a; Kalvass et al. 2007b; Kirschbaum et al. 2008). In the present study, we investigated the relevance of being a

substrate to P-gp in our mouse model by pharmacodynamic investigations in the open-field. The open-field test was chosen to detect several behavioral changes related to both wanted and unwanted effects of the drug. Our results demonstrate that venlafaxine has an impact on open-field behavior and this is modulated by P-gp activity. The impact on rodent behavior of venlafaxine treatment is in line with previous investigations of us (Kugelberg et al. 2005; Wikell et al., 2002) and others (Brocco et al. 2002; de Oliveira et al. 2004). Behavioral changes indicated an impact on exploration-related behaviors in the open-field (Gentsch et al. 1987; Schmitt et al. 1998; Swiergiel et al. 2007) as center activity was increased and rears were decreased by venlafaxine treatment. Those pharmacodynamic effects were even more pronounced in *abcb1ab (-/-)* mice 7 days after chronic treatment. Whether the increase in effect size 9 days after chronic drug administration, especially in *abcb1ab (+/+)* mice, has to be attributed to the time of treatment or a changed ratio between venlafaxine and its metabolites could not be answered by the present investigation. However, the chronic data revealed constant levels of Odm- and Ndm-venlafaxine which might contribute to the behavioral changes. Therefore, a pharmacodynamic analysis after pure metabolite treatment is of specific interest to solve those questions.

The strength of the present study is the combination of pharmacokinetic and pharmacodynamic effects of a chronic antidepressant treatment with respect to the impact of P-gp expression. However, there are some limitations in the analysis of the pharmacodynamic data that needs to be addressed. Repeated testing in an open-field setting leads to habituation (Thiel et al. 1999) which is seen in the present data by reduced overall activity in the test 9 days after treatment. Whether habituation is affected differentially in these genotypes is unknown so far. Similarly there is only little known about a specific influence of venlafaxine on habituation. However, there are some reports of venlafaxine affecting learning and memory in a positive way (Nowakowska et al. 2005; Nowakowska et al. 2003). Thus the

more pronounced effects of venlafaxine 9 days after chronic treatment were hardly explained by drug-mediated habituation processes.

In conclusion, our findings along with previous results show that P-gp at the BBB plays an important role in limiting brain entry of the enantiomers of venlafaxine and its metabolites. The P-gp substrate properties were reflected in the open-field test where the P-gp deficient mice displayed alterations in exploration activity compared with wildtype controls following the chronic drug exposure. Taken together, the present pharmacokinetic and pharmacodynamic findings offer the possibility that the expression of P-gp in patients may be a contributing factor for limited treatment response.

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Table 1 Concentrations (S+R, S and R) and enantiomeric ratios (S/R) of venlafaxine, O-desmethylvenlafaxine (Odm-venlafaxine), N-desmethylvenlafaxine (Ndm-venlafaxine) and N,O-didesmethylvenlafaxine (Ddm-venlafaxine) in *abcb1ab (-/-)* and *abcb1ab (+/+)* mice after chronic administration of racemic venlafaxine (10 mg/kg i.p. twice daily for 10 days, *n*=10 in each group).

	Serum			Brain		
	abcb1ab (-/-)	*abcb1ab (+/+)*	*P-value*	*abcb1ab (-/-)*	*abcb1ab (+/+)*	*P-value*
	mean ± SEM	mean ± SEM		mean ± SEM	mean ± SEM	
Venlafaxine						
Total (S+R)	1277 ± 82	1083 ± 44	*ns*	10500 ± 543	5156 ± 111	< 0.001
S-enantiomer	654 ± 42	575 ± 22	*ns*	5310 ± 278	3003 ± 72	< 0.001
R-enantiomer	623 ± 40	507 ± 22	< 0.05	5170 ± 265	2152 ± 43	< 0.001
S/R ratio	1.05	1.14	< 0.001	1.02	1.40	< 0.001
Odm-Venlafaxine						
Total (S+R)	183 ± 7.9	168 ± 6.9	*ns*	633 ± 18	300 ± 10	< 0.001
S-enantiomer	117 ± 5.1	108 ± 4.5	*ns*	390 ± 11	186 ± 6.5	< 0.001
R-enantiomer	66.7 ± 2.8	60.1 ± 2.4	*ns*	243 ± 7.0	114 ± 3.8	< 0.001
S/R ratio	1.75	1.80	< 0.05	1.60	1.64	*ns*
Ndm-Venlafaxine						
Total (S+R)	2341 ± 136	2067 ± 132	*ns*	7538 ± 188	2061 ± 71	< 0.001
S-enantiomer	1509 ± 88	1370 ± 87	*ns*	4322 ± 103	1339 ± 41	< 0.001
R-enantiomer	832 ± 49	697 ± 46	*ns*	3216 ± 89	722 ± 33	< 0.001
S/R ratio	1.82	1.97	< 0.01	1.35	1.87	< 0.001
Ddm-Venlafaxine						
Total (S+R)	18 ± 0.82	17 ± 1.0	*ns*	14 ± 0.46	5.88 ± 0.69	< 0.001
S-enantiomer	12.2 ± 0.57	11.2 ± 0.68	*ns*	9.0 ± 0.46	4.5 ± 0.52	< 0.001
R-enantiomer	6.0 ± 0.28	5.5 ± 0.35	*ns*	4.6 ± 0.17	2.2 ± 0.10	< 0.001
S/R ratio	2.03	2.04	*ns*	1.99	2.08	*ns*

Concentrations in nmol/L. *ns* = not significant. *P*-values < 0.05 indicate significant differences between *abcb1ab (-/-)* and *abcb1ab (+/+)* (unpaired two-tailed Student's *t*-test).

Figure legends

Fig. 1. Serum S/R concentration ratios (A) and brain S/R concentration ratios (B) of venlafaxine, O-desmethylvenlafaxine (Odm-venlafaxine), N-desmethylvenlafaxine (Ndm-venlafaxine) and N,O-didesmethylvenlafaxine (Ddm-venlafaxine) in *abcb1ab (-/-)* and *abcb1ab (+/+)* mice after chronic administration of racemic venlafaxine (10 mg/kg i.p. twice daily for 10 days). Values are means ± SEM (n=10).

Fig. 2. Brain/serum ratios of venlafaxine, O-desmethylvenlafaxine (Odm-venlafaxine), N-desmethylvenlafaxine (Ndm-venlafaxine) and N,O-didesmethylvenlafaxine (Ddm-venlafaxine) in *abcb1ab (-/-)* and *abcb1ab (+/+)* mice after chronic administration of racemic venlafaxine (10 mg/kg i.p. twice daily for 10 days). Values are means ± SEM (n=10). **p<0.01, ***p<0.001

Fig. 3. Open-field locomotor activity of *abcb1ab (-/-)* and *abcb1ab (+/+)* mice after 7 (A and C) and 9 days (B and D) of chronic venlafaxine treatment (10 mg/kg i.p. twice daily) indicated by total locomotion, i.e. total distanced moved (A and B), and peripheral locomotion, i.e. distanced covered in the periphery of the arena (C and D). The mice were investigated for 10 min. Values are means ± SEM (n=10-16). *p<0.05

Fig. 4. Open-field exploration activity of *abcb1ab (-/-)* and *abcb1ab (+/+)* mice after 7 (A and C) and 9 days (B and D) of chronic venlafaxine treatment (10 mg/kg i.p. twice daily) indicated by time spent in the center area (A and B) and rears made in the periphery of the arena (C and D). The mice were investigated for 10 min. Values are means ± SEM (n=10-16). *p<0.05

Fig. 1

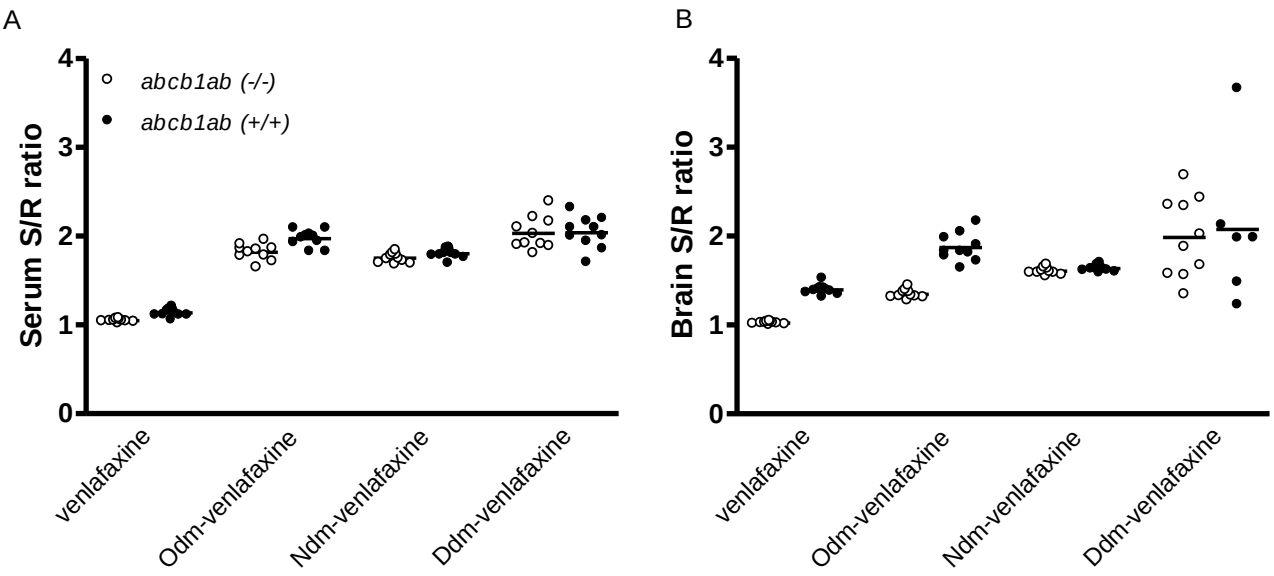
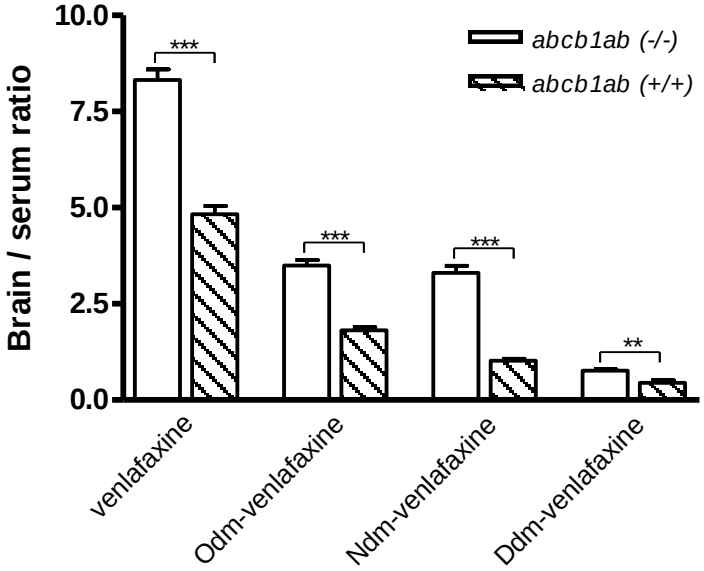

A
B
abcb1ab (-/-)
abcb1ab (+/+)
Serum S/R ratio
Brain S/R ratio
0
1
2
3
4
venlafaxine
Odm-venlafaxine
Ndm-venlafaxine
Ddm-venlafaxine

Fig. 2


10.0
7.5
5.0
2.5
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Brain / serum ratio
abcb1ab (-/-)
abcb1ab (+/+)
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venlafaxine
Odm-venlafaxine
Ndm-venlafaxine
Ddm-venlafaxine

Fig. 3

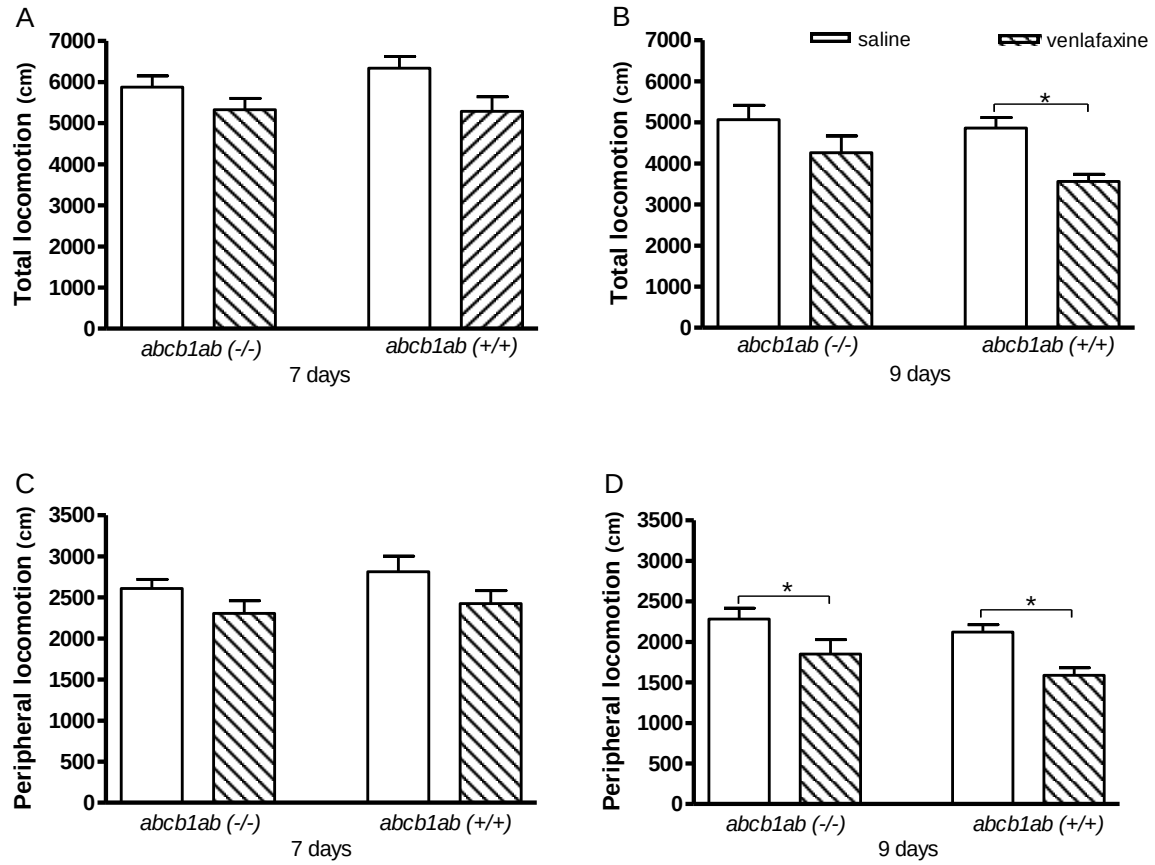
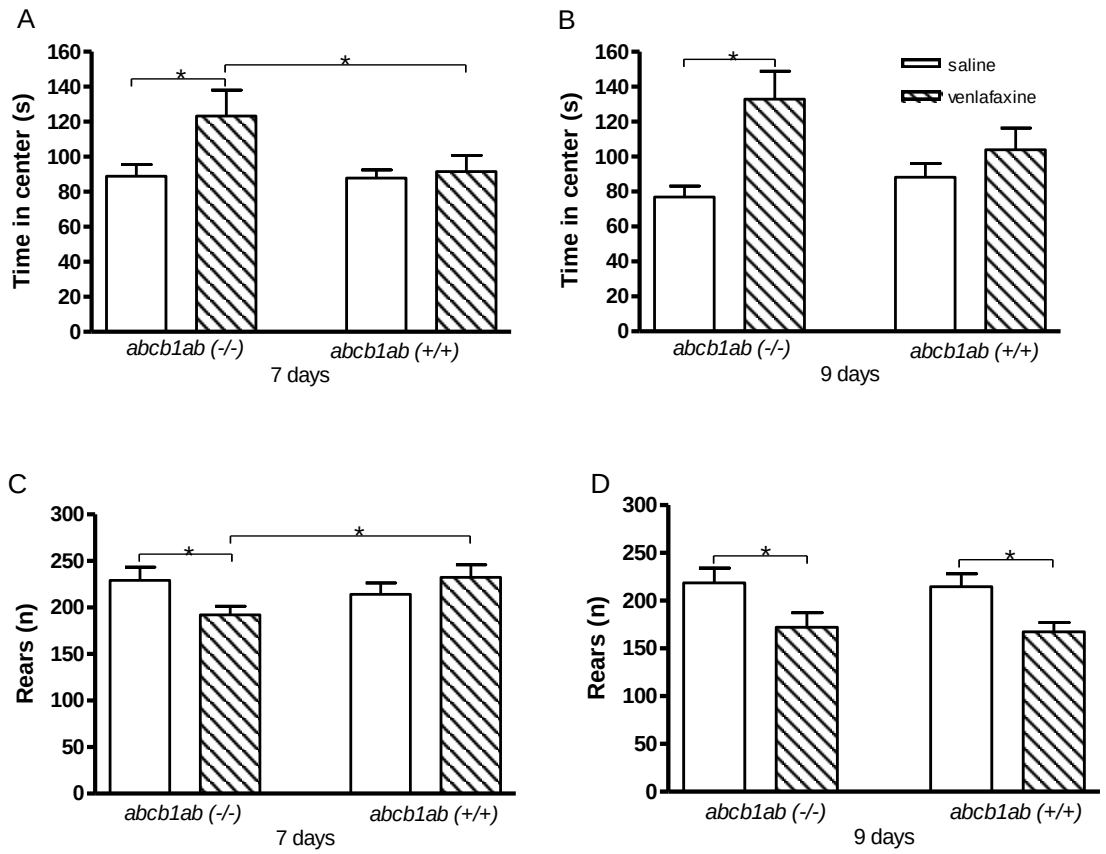

A
Total locomotion (cm)
7000
6000
5000
4000
3000
2000
1000
0
abcb1ab (-/-)
abcb1ab (+/+)
7 days
B
saline
venlafaxine
Total locomotion (cm)
*
abcb1ab (-/-)
abcb1ab (+/+)
9 days
C
Peripheral locomotion (cm)
3500
3000
2500
2000
1500
1000
500
0
abcb1ab (-/-)
abcb1ab (+/+)
7 days
D
Peripheral locomotion (cm)
*
*
abcb1ab (-/-)
abcb1ab (+/+)
9 days

Fig. 4


A
Time in center (s)
160
140
120
100
80
60
40
20
0
*
*
abcb1ab (-/-)
abcb1ab (+/+)
7 days
B
Time in center (s)
*
saline
venlafaxine
abcb1ab (-/-)
abcb1ab (+/+)
9 days
C
Rears (n)
300
250
200
150
100
50
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D
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