Journal of Psychiatric Research 109 (2019) 48–51

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

abcb1ab p-glycoprotein is involved in the uptake of the novel antidepressant vortioxetine into the brain of mice

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ARTICLE INFO

Keywords:
P-glycoprotein
abcb1
P-gp
Blood–brain barrier
Vortioxetine
Knock-out mice

ABSTRACT

A clinically important and well-studied transporter of the blood-brain barrier (BBB) is P-glycoprotein (P-gp), the gene product of *ABCB1*. Animal studies have shown that brain concentrations of many antidepressants depend on P-gp. However, biochemical properties, which might allow the prediction of pharmacodynamical involvement of P-gp have not yet been identified, hence thorough experimental testing of each novel drug is needed to determine its P-gp substrate status. In the current study, we tested the P-gp substrate status for the antidepressant vortioxetine using double *abcb1ab* knock-out (KO) mice. Cerebral concentrations of vortioxetine were 2.3 times higher in P-gp deficient mice compared to wildtype (WT) controls. No significant difference was found regarding the concentration of the drug in the plasma and other organs (liver, kidney, spleen) between KO and WT mice. The results of our study provide conclusive *in-vivo* evidence that in mice vortioxetine's brain bioavailability is P-gp dependent, expanding previous findings on this topic.

1. Introduction

For peripherally administered drugs acting on the central nervous system (CNS) such as antidepressants, passing the blood-brain barrier (BBB) and the final concentration within the brain proper are pivotal. A clinically important and well-studied energy-dependent membrane transporter protein of the BBB is the P-glycoprotein (P-gp), a member of the highly conserved superfamily of ATP-binding cassette (ABC) transporter proteins (Ambudkar et al., 1999; Brückl and Uhr, 2016; Cordon-Cardo et al., 1989; Thiebaut et al., 1987). The exact transport mechanism of P-gp is unknown, but it is assumed that P-gp recognizes substrate substances in the plasma membrane, binds to them and pumps them back into the blood circle. P-gp acts as specific active efflux pump for a wide range of compounds (Schinkel et al., 1996; Uhr and Grauer, 2003; Uhr et al., 2000, 2008) and it has been shown that antidepressants such as citalopram, venlafaxine, and amitriptyline are P-gp substrates (Doran et al., 2005). The human orthologue is encoded on chromosome 7 by the *ABCB1* gene (synonymous: multidrug resistance 1 (*MDR1*) (Callen et al., 1987). Next to the luminal membrane of brain capillary endothelial cells that form the BBB, P-gp is also expressed in other organs needing special protection, such as the placenta or testis (Brückl and Uhr, 2016).

Common polymorphisms in the human drug transporter-gene *ABCB1* alter the transporter's activity and hereby interfere with antidepressant treatment response (Breitenstein et al., 2015; Brückl and Uhr, 2016; Uhr et al., 2008), highlighting the clinical relevance of P-gp's pharmacodynamical impact. However, not all CNS-targeting drugs are substrates of P-gp. For example, for the antidepressant mirtazapine neither clinical investigations nor murine experimental data documented an influence of P-gp on clinical effectiveness or brain concentration respectively (Uhr et al., 2008). As common biochemical properties, which might allow the prediction of pharmacodynamical involvement of P-gp have not been identified so far, thorough experimental testing of each novel drug is needed to determine its P-gp substrate status. This information might be a prerequisite for further clinical investigation so that appropriate effective dosage for each patient might be individually adjusted.

Vortioxetine is a novel antidepressive compound. It is a 5-HT_{3A} 5-HT_7 and 5-HT_{1D} receptor antagonist, a partial 5-HT_{1B} agonist and an inhibitor of the serotonin transporter (SERT) (Alvarez et al., 2014).

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https://doi.org/10.1016/j.jpsychires.2018.11.009
Received 16 October 2017; Received in revised form 6 November 2018; Accepted 9 November 2018

Recently, a study showed vortioxetine to be a weak P-gp substrate *in vivo* in mice (Bundgaard et al., 2016), yet experiments were not conclusive solely showing a not statistically significant trend. Therefore, in this study we re-evaluate P-gp mediated effects on *in vivo* brain distribution of vortioxetine using P-gp deficient mice and P-gp competent wild-type counterparts.

2. Materials and methods

2.1. Materials and animals

Vortioxetine hydrobromide was obtained from Toronto Research Chemicals (Toronto, Canada). Male *abcab1ab(−/−)* mice and FVB/N wildtype mice were housed and maintained on a 12:12 h light/dark cycle (lights on at 07:00, with food and water ad libitum). *abcb1ab* double knockout mice, originally created by A. Schinkel by sequential gene targeting in 129/Ola E14 embryonic stem cells (Schinkel et al., 1996), and backcrossed seven times (N7), to FVB/N from the C57BL/6 × 129 chimera, and FVB/N wildtype mice were received from Taconic (Germantown, USA; FVB/Tac-[KO]Pgy2N7). An in-house homozygous colony is maintained at the Max Planck Institute of Psychiatry on the N7 FVB/N background through intercrossing of homozygous mice. Mice were 60 weeks old (standard error of the mean (SEM) = 4 weeks). Average body weight was 35 g (SEM = 3.9 g). All animal experiments were carried out in accordance with the Animal Rights Act of the State of Bavaria, which regulates the use and treatment of experimental animals. All housing and experimental procedures were in agreement.

2.2. Vortioxetine sample preparation and measurements

Vortioxetine hydrobromide was dissolved in 0.9% sodium chloride with 2.5% ethanol and was administered intraperitoneal (i.p.) (0.133 mg vortioxetine/kg bodyweight) after each animal was weighed. Injections took place between 09.00 and 12.00 a.m. One hour after injection, the mice were decapitated, trunk blood was taken and both the skull and the abdomen were surgically opened in order to remove the brain, liver, kidneys and spleen, which were washed in physiological NaCl solution and weighed and eventually homogenized in the fivefold volume phosphate buffered saline (PBS), containing “Complete Protease Inhibitor Cocktail Tablets” (Roche, Penzberg, Germany) using a Dispomix Drive (Medic Tools AG, Zug, Switzerland). The blood plasma and the brain homogenates were analysed using the combined high-performance liquid chromatography/mass spectrometry (HPLC/MS-MS) technique. Analysis was performed using an Agilent 1100 Series (Agilent, Waldbronn, Germany) liquid chromatograph which was interfaced to the ESI source of an Applied Biosystems API 4000 (ABSciex, Darmstadt, Germany) triple quadrupole mass spectrometer. All samples were prepared using Ostro protein precipitation and phospholipid removal plates (Waters, Eschborn, Germany). Deuterated vortioxetine (vortioxetine hydrobromide-D8) was used as internal standard. Chromatography was accomplished using a gradient elution in a Accucore RP-MS 2.6 µm column (2.1 × 50 mm, Thermo Scientific, Dreieich, Germany) at a flow rate of 0.3 ml/min and 30 °C. The composition of eluent A was methanol with 10 mM ammonium formate with 0.1% formic acid and water with 10 mM ammonium formate with 0.1% formic acid as eluent B. The gradient was 0–0.5 min 55% A, 0.5–2 min 55–90% A, 2–3 min held at 90% A, 3–3.5 min 90-55% A and 3.5–8min 55% A. The total run time was 8 min and the injection volume was 5 µl. The retention time for vortioxetine and deuterated vortioxetine was 4.1 min. The ion source was operated in the positive mode at 500 °C, and multiple reaction monitoring (MRM) collision-induced dissociation (CID) were performed using nitrogen gas as the collision gas. The collision energy was set to 35 V for vortioxetine and 35 V for deuterated vortioxetine. The transitions monitored during analysis were m/z 299.4 → 150.1 for vortioxetine and m/z 307.2 → 153.0 for Vortioxetine-D8. The detection limit for vortioxetine in plasma was 30 ng/ml and 18 ng/g wet weight in brain tissue.

2.3. Statistical analysis

Differences in the plasma and organ concentrations of vortioxetine between the mutants and the wild-type animals were tested for significance by one-factorial multivariate analyses of variance (MANOVA). Each group consisted of 8 mice. The plasma and organ concentrations were the dependent variables and group, a between-subjects factor with two levels (mutants vs. wild-type mice), was the independent variable. When a significant group effect was found for an organ sample or plasma, univariate F-tests followed to identify the variables, whose differences between the two groups contributed significantly to the global group effect. As level of significance $\alpha = 0.05$ was accepted; p values were corrected according to the Bonferroni procedure for all a posteriori tests (univariate F-tests) in order to keep the type I error less than or equal to 0.05.

3. Results

We used previously described mouse mutants lacking the homologs of the human *ABCB1* gene (i.e., *abcb1ab* double knockout mice) as an *in-vivo* assay (Uhr et al., 2008) to investigate the brain bioavailability of the novel antidepressant vortioxetine. One hour after one single intraperitoneal administration of the antidepressant (0.133 mg/kg bodyweight) we observed that the intracerebral concentration of vortioxetine was 2.3 higher in the mutant mice compared to the P-gp competent wild-type controls (n = 8 animals per each group; MANOVA; $p < 0.05$; Table 1). No significant changes of vortioxetine concentration in spleen, liver, or kidney were found between *abcb1ab* (−/−) mutants and wild-type mice. In Fig. 1, the blood-organ barrier function is represented as an organ/plasma concentration ratio. For each organ the identified ratio in the wild-type (WT) mice was set to 100% respectively. Furthermore, no differences in vortioxetine plasma concentrations were found between *abcb1ab* (−/−) mutants and WT mice.

4. Discussion

In this study, we show conclusive differences of the cerebral concentrations of vortioxetine between *abcb1ab* (−/−) double knock out mice and wildtype (WT) mice. These differences suggest that vortioxetine is a substrate of *abcb1ab* P-glycoproteins and P-gp might play an intergral role in the transport of vortioxtine across the BBB in the brain of mice. In the animals lacking P-gp, the penetration of vortioxetine

Table 1
Organ concentrations of vortioxetine 1 h after intraperitoneal injection in both *abcb1ab* (−/−) and wild-type (WT) mice.

	mean	abcb1ab(−/−)	mean	WT	ratio	*P*-value
		SEM		SEM		
plasma	181,29	12,76	188,26	25,03	0,96	n.s.
cerebrum	175,97	14,08	84,65	17,07	2,07	*
spleen	240,17	25,03	200,76	42,19	1,19	n.s.
liver	97,26	16,09	88,63	17,98	1,09	n.s.
kidney	210,77	17,26	252,65	46,96	,83	n.s.
Cer_Pla	,97	,04	,42	,05	2,32	*
Spl_Pla	1,34	,12	,97	,12	1,38	n.s.
Liv_Pla	,52	,06	,44	,05	1,19	n.s.
Kid_Pla	1,17	,08	1,27	,11	,92	n.s.

Values are mean ± SEM (standard error of the mean). Plasma concentration are given in ng/ml, organ concentrations in ng/g moist mass. n.s. = not significant. *p < 0.05 (MANOVA). Ratios between plasma and brain, spleen, liver and kidney are given respectively (Cer_Pla, Spl_Pla, Liv_Pla and Kid_Pla).

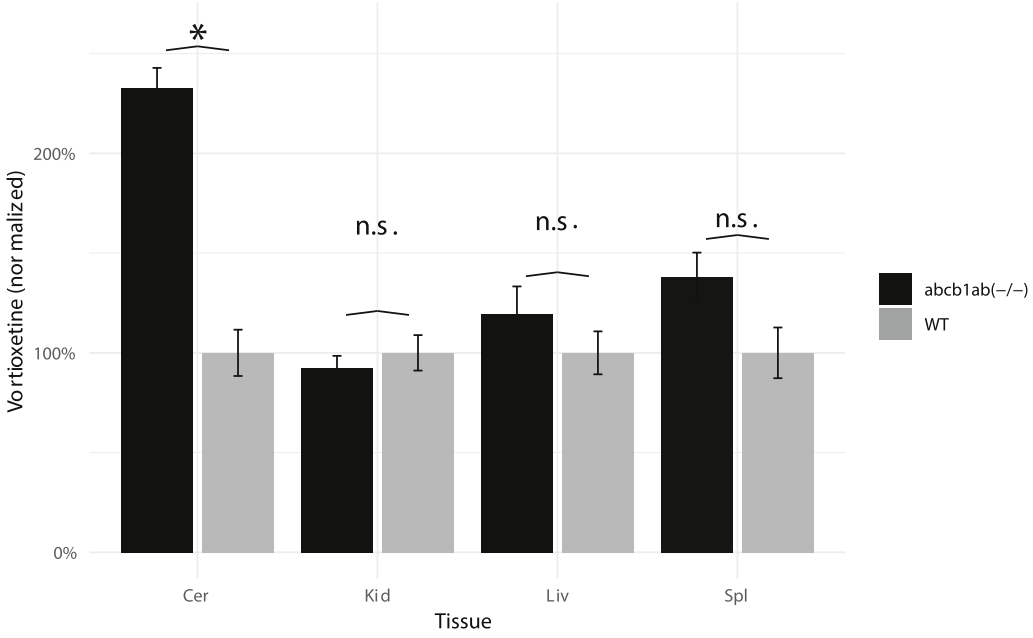

Fig. 1. Blood-organ barrier function for vortioxetine in mice.

into the brain is 2.3 times higher than in control animals. Vortioxetine fits in the group of other antidepressive compounds previously shown to be P-gp dependent with similar brain/plasma concentration ratios (citalopram: 3.7, venlafaxine: 1.8, and d-venlafaxine: 3.6) (Uhr et al., 2008; Doran et al., 2005). Vortioxetine concentrations did not differ significantly in spleen, liver and kidney comparing *abcb1ab (−/−)* double KO and WT animals. Plasma concentrations also showed no significant differences. This supports the hypothesis that a distinct group of antidepressants such as vortioxetine are exported against a concentration gradient from the intracerebral into the extracerebral space via the BBB and that the observed effect is specific for the transport across the BBB and not due to passive mechanisms.

An *in-vivo* study that recently analysed P-gp dependent brain bioavailability of vortioxetine found rather less definite results and drew the conclusion that vortioxetine is, if at all, merely a weak P-gp substrate in mice (Bundgaard et al., 2016). Several reasons might be responsible for the diverging results. As Bundgaard et al. (2016) applied 3.8x more vortioxetine (0.5 mg/kg body weight s.c. vs. 0.133 mg/kg body weight i.p.) the high dosages might have overridden any inhibiting P-gp effects. Next, it is noteworthy that the method the drug was administered was different. While we used single intraperitoneal injections, they used subcutaneous injections administered both at a single time point and continuously (72 h). Intraperitoneal injections differ from subcutaneous drug administration with regard to differences in first-pass effect and disparate velocities of bioavailability. It has been demonstrated that the administration technique can be essential and intraperitoneal and subcutaneous injections can yield even contradictory results, as it was the case in corresponding studies focusing on the antidepressant amitriptyline (Grauer and Uhr, 2004; Uhr et al., 2000, 2007). Furthermore, two distinct *in-vivo* models were applied: on the one hand *abcb1ab* (−/−) double knock out mice (Schinkel et al., 1996), on the other hand mdr1a-deficient mice (Charles River Laboratories (Wilmington, MA, USA) (Bundgaard et al., 2016). Pharmacokinetics might be different in *abcb1a* single knock out compared to *abcb1ab* (−/−) double knock out mice (Brückl and Uhr, 2016). The endothelial cells constituting the BBB express both abcb1a and abcb1b (to a lesser degree), and a large overlap exists in the spectrum of compounds transported by these two different P-gps (Uhr et al., 2007). Thus, a compensatory upregulation of *abcb1b* in the single *abcb1a* KO mice used by Bundgaard et al. (2016) or a potentially higher affinity of vortioxetine to *abcb1b* could further explain the less pronounced P-gp effects observed in the previous study.

Basically, many substrate affinities of P-gp seem to be conserved between species. For many compounds this conservation was shown both in clinical trials and experimental animal studies (Chu et al., 2013; Feng et al., 2008; Uhr et al., 2008). Thus, the brain bioavailability of vortioxetine due to P-gp affinity observed in this study using mice might have an impact on antidepressive treatment in humans too. Specific genomic variation (single nucleotide polymorphisms; SNP) in the human *ABCB1* gene has been shown to alter either the expression or activity of P-gp in humans (Uhr et al., 2008). Given a possible P-gp involvement on vortioxetine in humans that is based on our results in rodents, *ABCB1* genomic variation in humans might alter the extent of brain distribution of vortioxetine. However, some P-gp substrate affinities are reported to exhibit no similiar pattern between species (Katoh et al., 2006; Yamazaki et al., 2001), so that in the end only a clinical trial is able to elucidate a putative clinical impact for psychiatric treatment based on our finding in rodents. In order to substantiate the rationale for such clinical investigation, additional functional murine data might support the effect beforehand. In this line enhanced serotonin (5-HT) levels, higher target occupancy in *abcb1ab* (−/−) mice or behavioral tests are interesting topics to be studied.

In conclusion, this study provides conclusive *in-vivo* evidence that vortioxetine's brain bioavailability is P-gp dependent in mice. It might initiate further, in particular clinical studies investigating the brain bioavailability in humans with respect to the genomic *ABCB1* variation (Siddiqui et al., 2003; Uhr et al., 2008). From the clinical point of view, information on the P-gp dependency of the brain bioavailability of a centrally acting drug are essential for individual dosage recommendations and, therefore, should also be considered for official approval procedures.

Organ/plasma ratios of vortioxetine concentrations in *abcb1ab* (−/−) mice compared to wild-type (WT) controls after one single intraperitoneal administration. The organ/plasma ratios of vortioxetine are shown as percentage of the control which was set for 100%. An asterisk indicates a significant difference between the knockout mutants and the control mice (univariate F tests in MANOVA), p value < 0.05). Cerebrum (Cer), spleen (Spl), kidney (Kid), liver (Liv) were investigated. Values are shown as means ± SEM (standard error of the mean).

Conflicts of interest

None.

Role of funding resources

This work was funded by the Max-Planck-Society.

CRediT authorship contribution statement

Derek Spieler: Conceptualization, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Christian Namendorf:** Investigation. **Tamara Namendorf:** Investigation. **Manfred Uhr:** Conceptualization, Formal analysis, Visualization, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Acknowledgements

We are grateful to Matthias Braeunig for his support in creating the figure.

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