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Induction of Drug Efflux Protein Expression by Venlafaxine but not Desvenlafaxine

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ABSTRACT: Venlafaxine and its metabolite desvenlafaxine are serotonin-norepinephrine reuptake inhibitors currently prescribed for the treatment of depression. Previously, it was reported that venlafaxine is an inducer of MDR1, the gene responsible for P-glycoprotein (P-gp). The present study expanded upon these findings by examining the effect of venlafaxine and desvenlafaxine on the expression of both P-gp and the breast cancer resistance protein (BCRP) in human brain endothelial cells (HBMEC), an *in vitro* model of the blood–brain barrier (BBB). The HBMEC were treated for 1 h with various concentrations (500 nM to 50 μM) of venlafaxine and desvenlafaxine. Western blot analysis revealed treatment with venlafaxine significantly induced the expression of P-gp (2-fold) and BCRP (1.75-fold) in a dose-dependent manner, while treatment with desvenlafaxine had no effect on drug efflux transporter expression. To determine the functional significance of this effect, the permeability of a known drug efflux probe, rhodamine 123, across the BBB model and Caco-2 cells, a model of intestinal absorption, were examined. Treatment with venlafaxine (1–50 μM) for 1 h significantly reduced the apical-to-basolateral permeability of R123 across the BBB model (30%) and Caco-2 cell monolayers (25%), indicative of increased drug efflux transporter expression at the apical membrane. Conversely, desvenlafaxine had no effect on R123 permeability in either cellular model. These studies indicate that venlafaxine, but not desvenlafaxine is an inducer of drug efflux transporter expression, which consequently increases the potential for clinical drug–drug interactions. Therefore, based on these preliminary results, caution should be taken when prescribing venlafaxine with other P-gp substrates. Copyright © 2011 John Wiley & Sons, Ltd.

Key words: venlafaxine; desvenlafaxine; P-glycoprotein (P-gp); breast cancer resistance protein (BCRP); blood–brain barrier (BBB)

Introduction

P-glycoprotein (P-gp) is a 170 kDa protein derived from the multidrug resistance gene, MDR1 (ABCB1), and a member of the ATP-binding cassette (ABC) superfamily of transmembrane proteins. A more recent addition to the ABC family is the 72 kDa breast cancer resistance protein (BCRP), a product of the BCRP (ABCG2) gene. These proteins are responsible for the active efflux, through ATP hydrolysis, of a vast range of structurally unrelated compounds and peptides [1]. These transport systems are recognized for their ability to confer a multidrug resistant phenotype, most notably in cancer cells [2,3] and bacteria [4,5]. These proteins are also present in normal cells where they protect sensitive tissue

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(e.g. brain, testis and fetus) from cytotoxicity and facilitate the elimination of xenobiotics and metabolites from the body (for a complete review see [6]). The presence of P-gp and BCRP in the intestinal epithelium, liver hepatocytes, proximal tubules of the kidney and brain endothelium, in particular, can dramatically influence the pharmacokinetic profile of drugs that interact with these proteins [6,7].

Venlafaxine (Effexor®) and its metabolite, desvenlafaxine (Pristiq®), are serotonin-norepinephrine reuptake inhibitors currently FDA labeled for the treatment of major depressive disorder (venlafaxine is also FDA labeled for a number of other uses). Recently, studies have begun to evaluate the drug efflux interactions of these therapies. Uhr *et al.* concluded that both venlafaxine and desvenlafaxine were substrates for P-gp based on their elevated concentrations in the brains of P-gp knockout mice compared with P-gp-expressing wild-type controls [8]. Similar *in vivo* observations were reported for the enantiomers of venlafaxine and its major metabolites, including desvenlafaxine [9]. With respect to inhibition, Weiss *et al.* determined that venlafaxine, but not desvenlafaxine, inhibited P-gp activity *in vitro*, though the effect observed with venlafaxine was categorized as only weakly inhibitory [10]. A more recent study reinforced these claims demonstrating that venlafaxine and desvenlafaxine exhibit only modest inhibition of P-gp function with IC_{50} values exceeding 250 μM [11]. The authors further state that neither of these drugs act as substrates for P-gp based on their low efflux ratios in cells that express P-gp [11], which contradicts the aforementioned *in vivo* observations using P-gp knockout animals [8,9]. Clearly, more studies are required adequately to address the drug efflux ramifications of these antidepressant medications.

Despite recent efforts to decipher the drug efflux interactions of venlafaxine and desvenlafaxine, there is a noticeable lack of data describing the impact of these drugs on efflux protein expression and, to our knowledge, no study to date has investigated the interactions of either of these drugs with BCRP. This is important as drug efflux transporter induction often results in drug–drug interactions that can dramatically alter the oral bioavailability and tissue distribution of a drug. For example, concomitant administration of the known drug efflux inducer, rifampin, substantially reduced digoxin and talinolol plasma concentrations after oral administration; an effect attributed to increased P-gp expression in the intestine by rifampin [12–14]. Similarly, induced drug efflux transporter expression at the blood–brain barrier (BBB) has been shown significantly to reduce the brain penetration of known substrates [15].

Previously it was reported that Caco-2 cells, an intestinal epithelial cell line, treated with venlafaxine resulted in a significant induction of MDR1 gene expression [16]. The present study expands upon these findings examining the effect of not only venlafaxine, but also desvenlafaxine, on the expression of both P-gp and BCRP in human brain endothelial cells, an *in vitro* model of the blood–brain barrier (BBB). In addition, this study demonstrates the functional significance of the observed changes in drug efflux transporter expression by examining the permeability of a known drug efflux probe, rhodamine 123 (R123), across both the *in vitro* BBB model and Caco-2 cells. Here, treatment with venlafaxine significantly induced P-gp and BCRP expression in brain endothelial cells and reduced the penetration of R123 across the BBB model and Caco-2 cell monolayers. Conversely, desvenlafaxine had no effect on drug efflux transporter expression or function in the cellular models. These studies indicate that venlafaxine, but not desvenlafaxine, is an inducer of efflux protein expression, which opens the door to potential drug–drug interactions in a clinical setting. As such, caution should be taken when prescribing this antidepressant medication.

Materials and Methods

Materials

Human brain microvascular endothelial cells (HBMEC), endothelial cell media (ECM), endothelial cell growth supplement (ECGS), fetal bovine serum and penicillin/streptomycin solution were purchased from Sciencell Research Laboratories (Carlsbad, CA). Human epithelial colorectal adenocarcinoma cells (Caco-2), Eagle's minimum essential medium (EMEM) and fetal

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bovine serum were purchased from American Type Culture Collection, ATCC (Manassas, VA). Venlafaxine and desvenlafaxine were provided by Wyeth Pharmaceuticals (now a part of Pfizer). Fibronectin solution, rifampicin and rhodamine 123 (R123) were purchased from Sigma Chemical Co. (St Louis, MO). The mouse monoclonal antibody for P-glycoprotein, P-gp (C219), was purchased from EMD Chemicals Inc. (Hawthorne, NY). The antibodies for the breast cancer resistance protein, BCRP (BXP-21) and actin (clone C4) were purchased from Millipore (Temecula, CA). The 24-well membrane inserts (translucent, 0.4 μm pore), 24-well companion plates and 6-well plates were purchased from Fisher Scientific (St Louis, MO).

Cell culturing

The HBMEC culture media (ECM) was supplemented with 5% fetal bovine serum, 1% penicillin/streptomycin solution and 1% growth supplement (ECGS) while the Caco-2 culture media (EMEM) was supplemented with 20% fetal bovine serum. All cells were cultured in a humidified 37°C incubator with 5% CO_2. Media replacement was carried out every other day until the monolayers reached confluence (3–4 days).

Time course

Stock solutions of rifampicin, venlafaxine and desvenlafaxine were prepared by dissolving each drug in nanopure water. The HBMEC were seeded at 100000 cells/cm^2 onto fibronectin-coated (1 μg/cm^2), 6-well (9.6 cm^2/well) plates. The cells (at 80–90% confluence) were exposed to each drug (50 μM) for 1, 3, 6, 24, 48 and 72 h in a manner similar to that described by us previously [16]. Following the treatment period, the cells were washed with PBS and each well was exposed to 150 μl of ice-cold M-PER reagent (Pierce Biotechnology, Rockford, IL) containing phenylmethanesulfonyl fluoride (1 mM) and Halt Protease and Phosphatase Inhibitor Cocktail (1X) (Thermo Scientific, Waltham, MA). Samples were placed at −80°C until collection.

Toxicity

Cellular toxicity was evaluated using a cytotoxicity detection kit from Roche Diagnostics (Indianapolis, IN). This assay quantifies cell death and cell lysis based on the colorimetric measurement of lactate dehydrogenase (LDH) released from the cytosol of damaged cells into the extracellular supernatant. Following each treatment period in the time course studies, the extracellular media from each group was collected and evaluated for LDH activity according to the manufacturer's protocol.

Dose-response

These studies were performed using the same procedure described for the time course experiments. Once again, HBMEC were seeded onto 6-well plates and, upon confluency, were exposed to various concentrations (0, 0.5, 1, 5, 10 or 50 μM) of venlafaxine or desvenlafaxine for 1 h at 37°C and 5% CO_2. As described above, the cells were washed, exposed to lysis buffer, and placed at −80°C for storage.

Western blot

Western blot studies were performed as previously described by our group [17]. Briefly, the cell lysates from each group were collected and evaluated for protein content using the bicinchoninic acid (BCA) method (Thermo Scientific, Waltham, MA). Samples in Laemmli Buffer (Bio-Rad, Hercules, CA) were loaded (50 μg of protein) onto a Criterion 4–20% Tris-HCl gradient gel (Bio-Rad, Hercules, CA). Migration transpired using 50–130 V over a 2 h period. Following migration, electrotransfer to an Immun-Blot PVDF (polyvinylidene fluoride) membrane occurred overnight at 4°C and 90 mA. The membrane was immunoprobed with mouse monoclonal antibodies for P-gp (1:500 dilution), BCRP antibody (1:2000 dilution) and the housekeeping protein actin (1:1000 dilution) in 5% blotting-grade blocker (nonfat dry milk) (Bio-Rad, Hercules, CA) for 3 h. The membrane was washed with deionized water and exposed to HRP-linked mouse secondary antibody (Cell Signaling Technology, Danvers, MA) for 1 h. Following a 30 min wash in deionized water, the membrane was revealed using SuperSignal West Femto maximum sensitivity substrate (Thermo Scientific, Waltham, MA) and exposed with a Bio-Rad ChemiDoc XRS molecular imager (Bio-Rad, Hercules, CA).

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Permeability

These studies were performed using a human *in vitro* model of the BBB, previously characterized by our group [17], and Caco-2 cells, an *in vitro* model of the human intestine. Here, HBMEC were seeded at 100000 cells/cm^2 onto fibronectin-coated (4 μg/cm^2), 24-well, 0.4 μm-pore, translucent membrane inserts (0.3 cm^2/insert) to establish a polarized monolayer representative of the BBB. The layer of endothelial cells separates this system into an apical ('blood' side) and basolateral ('brain' side) compartment. The Caco-2 cells were seeded at 75000 cells/cm^2 onto non-coated 24-well inserts. Cells were treated with the same concentrations (0, 1, 5, 10 or 50 μM) of venlafaxine or desvenlafaxine for the same period of time (1 h) as that established in the dose-response studies. Following the 1 h treatment, the medium was removed and fresh medium containing 5 μM R123 was introduced to the apical side of each cellular model, while blank medium was placed in the basolateral compartment. The apical compartment was sampled at time 0 to establish the initial concentration of R123 in each group. Following exposure of the inserts containing R123 to the wells with blank medium, the cells were incubated at 37°C. Samples were collected from the basolateral compartment at various time points (0, 30, 60, 90 and 120 min) to assess the permeability of R123 across the cell monolayer (apical to basolateral). The samples were analyzed (λ ex = 485 nm and λ em = 516 nm) for R123 content using a BioTek Synergy HT multi-detection microplate reader (Winooski, VT). The apparent permeability (*P*app) of R123 was calculated using the equation *P*app $1/AC_0$ * (dQ/dt), where A represents the surface area of the membrane, C_0 is the initial concentration of R123 in the apical compartment, and dQ/dt is the amount of R123 that appears in the basolateral compartment in the given time period [18]. The *P*app of R123 across the cell monolayer under each of the treatment conditions was expressed as a percentage of control.

Statistics

Statistical analyses were performed using an ANOVA and Bonferroni post-hoc test.

Results

Time course

As a follow up to our previous report that venlafaxine induces P-gp at the genomic level in Caco-2 cells [16], the effect of venlafaxine and its metabolite desvenlafaxine on P-gp expression at the protein level in human brain endothelial cells (HBMEC) was investigated. Due to growing interest in BCRP over the past decade and its ever expanding role in the disposition of drugs in the body, this study examined the effect of venlafaxine and desvenlafaxine on the expression of BCRP in these same cells, as well. As a positive comparison, the cells were also exposed to a known inducer of P-gp [19] and BCRP [20], rifampicin. For these studies a dose known to induce drug efflux transporter expression levels (50 μM) was used, based on our previous work [16], to establish the time course in which venlafaxine and desvenlafaxine alter protein expression.

The expression of P-gp and BCRP in the HBMEC following treatment with rifampicin, venlafaxine or desvenlafaxine at various time points up to 72 h is displayed in Figure 1. P-gp (Figure 1A) and BCRP (Figure 1B) expression for each treatment group was quantitated, normalized to beta-actin and expressed as a percentage of control. At the early time points (1 and 3 h), expression of both proteins increased upon exposure to venlafaxine (50 μM) and to a lesser extent desvenlafaxine (50 μM) when compared with the control. At the longer exposure periods (48 and 72 h), protein expression in the treatment groups was similar to or slightly lower than the control. The expression profiles in the presence of venlafaxine over time were comparable to that observed with 50 μM rifampicin, a known inducer of P-gp and BCRP (Figure 1). A peak in the expression of both P-gp and BCRP was observed around 1 h. Figure 2 displays representative western blots depicting the expression of P-gp (Figure 2A), BCRP (Figure 2B), and the housekeeping protein actin in the HBMEC following the 1 h treatment. Treatment with rifampicin and venlafaxine significantly elevated the expression of both P-gp and BCRP. While treatment with desvenlafaxine also increased the

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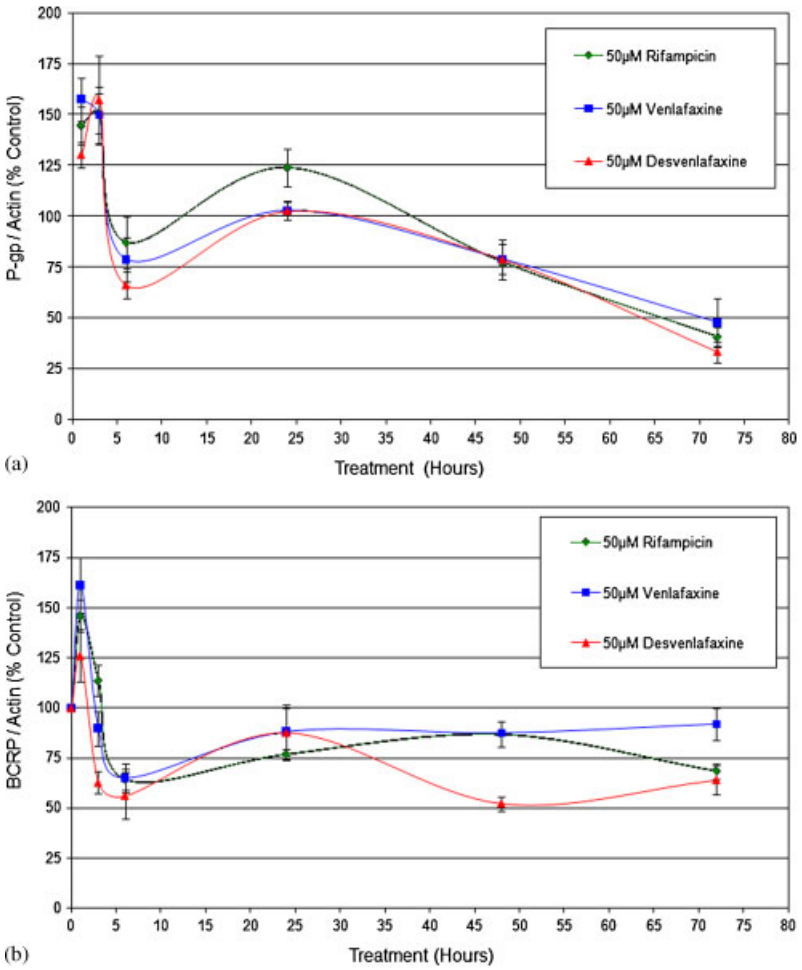

Figure 1. Expression of (a) P-gp and (b) BCRP following treatment (50 μM) with venlafaxine, desvenlafaxine or rifampicin in the HBMEC for 1, 3, 6, 24, 48 or 72 h. P-gp and BCRP expression was determined through immunoblot, quantitated via densitometric analysis and normalized to actin. Values represent the mean ± SEM of four separate western blot experiments and are displayed as a percentage of control

expression of these proteins over the control, these effects did not reach statistical significance. The effect of each treatment on P-gp and BCRP expression in the HBMEC at 1 h is summarized in Table 1.

Toxicity

An evaluation of the cellular toxicity, as determined using a LDH cytotoxicity kit, following treatment with 50 μM venlafaxine or desvenlafaxine is presented in Figure 3. There was no difference in the appearance of LDH in either drug-treated group compared with the control.

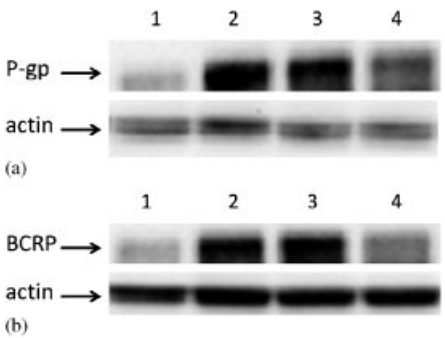

Figure 2. Representative immunoblots of (a) P-gp and (b) BCRP with their respective actin bands. HBMEC were treated (50 μM) with control (lane 1), rifampicin (lane 2), venlafaxine (lane 3) or desvenlafaxine (lane 4) for 1 h prior to evaluation via western blot

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Dose-response

After determining the time frame in which venlafaxine and desvenlafaxine influence P-gp and BCRP expression, the study examined whether these effects could be observed at doses lower than 50 μM, as these concentrations are likely more relevant clinically. Using the peak time of 1 h established in the time course studies, the expression of P-gp and BCRP was examined following exposure to various concentrations of venlafaxine and desvenlafaxine in the HBMEC. Treatment with venlafaxine demonstrated a dose-dependent increase in the expression of both P-gp (Figure 4) and BCRP (Figure 5), inducing levels to nearly twice that observed for control. More notably, these effects were statistically significant at doses as low as 1 μM (Figure 5). The effect of desvenlafaxine on drug efflux transporter expression was less dramatic than that observed for venlafaxine. While P-gp expression did increase in a dose dependent manner in the presence of desvenlafaxine compared with the control, this effect did not reach statistical significance (Figure 4B). No change in BCRP expression was observed at any dose of desvenlafaxine in comparison with the control (Figure 5B).

Table 1. P-gp and BCRP expression following drug treatment in the HBMEC for 1 h. Values represent mean ± SEM ($n = 4$) and are displayed as a percentage of control. n.s., not significant, as determined by ANOVA and the Bonferroni post hoc test

		HBMEC	
		1 h	Significance
P-gp	Control	100 ± 11.8	—
	Rifampicin	144 ± 9.3	$p < 0.05$
	Venlafaxine	157 ± 10.6	$P < 0.01$
	Desvenlafaxine	130 ± 6.1	n.s.
BCRP	Control	100 ± 14.2	—
	Rifampicin	146 ± 8.0	$P < 0.05$
	Venlafaxine	161 ± 13.9	$P < 0.01$
	Desvenlafaxine	126 ± 12.9	n.s.

Permeability

To evaluate the functional impact of the induced drug efflux transporter expression observed in the presence of venlafaxine and desvenlafaxine, we examined the permeability of a well characterized P-gp [21,22] and BCRP [23] substrate, R123, in both the *in vitro* BBB model and Caco-2 cells. Treatment with venlafaxine for 1 h significantly decreased the apical-to-basolateral permeability of R123 in the BBB model at all of the concentrations examined, the lowest being

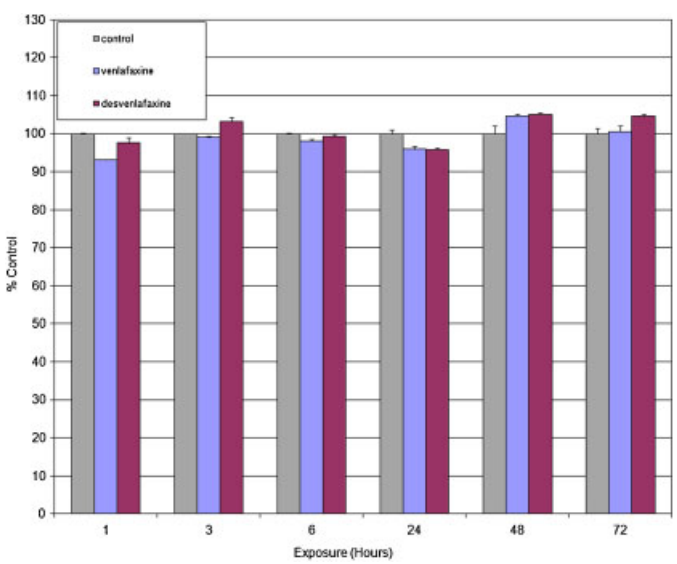

Figure 3. Appearance of LDH in the extracellular media following treatment (50 μM) with venlafaxine, or desvenlafaxine in the HBMEC for 1, 3, 6, 24, 48 or 72 h. Values represent the mean ± SEM ($n = 3$) and are displayed as a percentage of control. No significant difference as determined by ANOVA and Bonferroni post-hoc test

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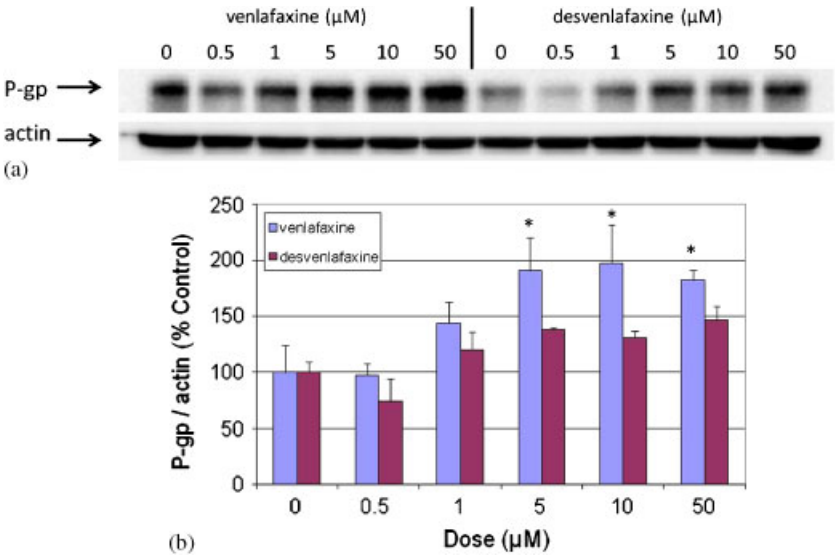

Figure 4. (a) Representative immunoblot of P-gp following treatment with venlafaxine or desvenlafaxine in the HBMEC. Various drug concentrations (0.5–50 μM) were exposed to the HBMEC for 1 h. (b) P-gp expression was quantitated via densitometric analysis and normalized to actin. Values represent the mean ± SEM of two separate western blot experiments. $^{*}p < 0.05$ as determined by ANOVA and Bonferroni post-hoc test

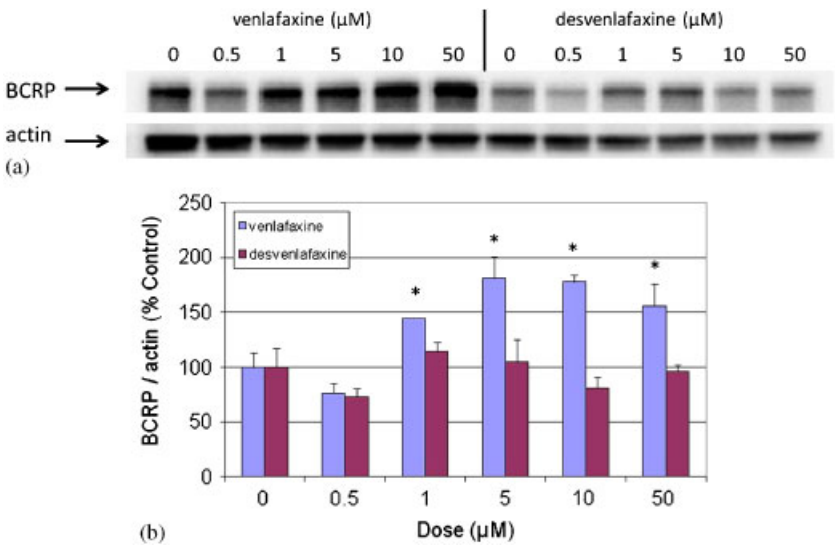

Figure 5. (a) Representative immunoblot of BCRP following treatment with venlafaxine or desvenlafaxine in the HBMEC. Various drug concentrations (0.5–50 μM) were exposed to the HBMEC for 1 h. (b) BCRP expression was quantitated via densitometric analysis and normalized to actin. Values represent the mean ± SEM of two separate western blot experiments. $^{*}p < 0.05$ as determined by ANOVA and Bonferroni post-hoc test

1 μM. The greatest effect was observed with 10 μM, where the 'brain' penetration of R123 was reduced by 30% (Figure 6A). While the effect of 50 μM venlafaxine on R123 permeability was statistically lower than the control, the magnitude of this effect was less dramatic than that observed with 5 μM or 10 μM venlafaxine (Figure 6A). Conversely, treatment with the same

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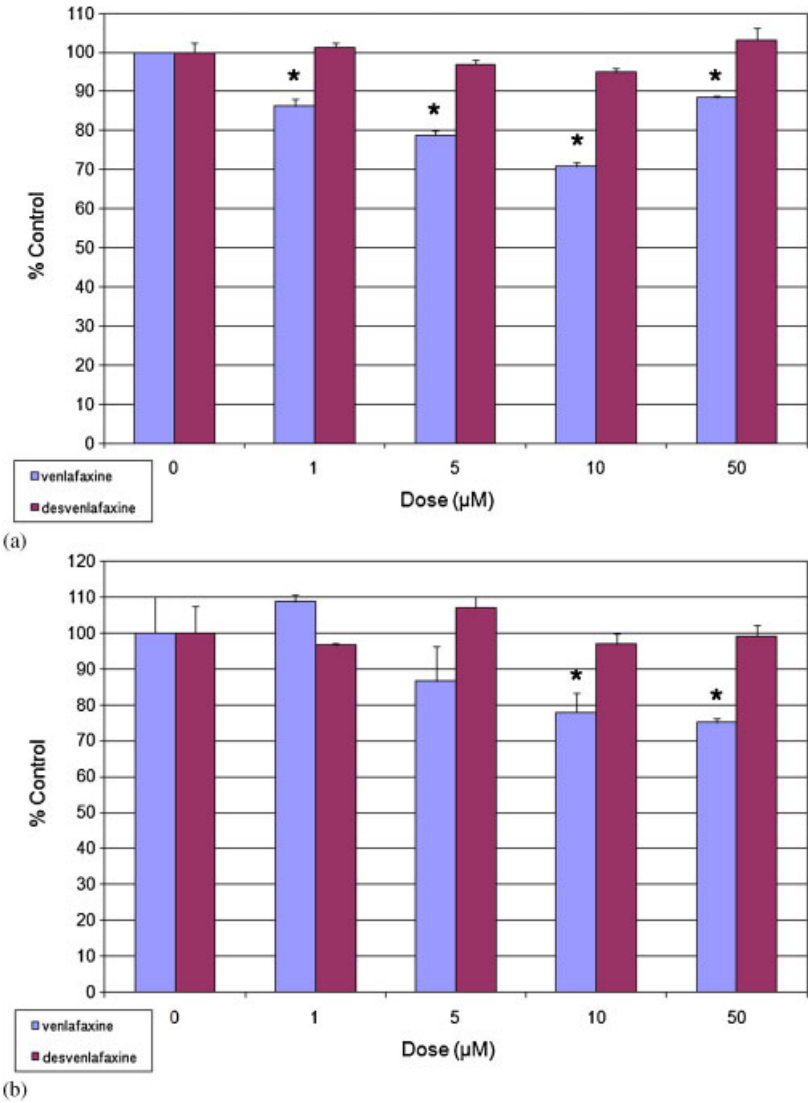

Figure 6. Apical-to-basolateral permeability of R123 in (a) the HBMEC *in vitro* model of the BBB and (b) Caco-2 monolayers following treatment with venlafaxine or desvenlafaxine. Various concentrations of drug (1–50 μM) were exposed to the apical compartment for 1 h. The drug-containing media was removed and replaced with 5 μM R123 in fresh media. Samples were collected from the basolateral compartment every 30 min for 2 h to examine R123 permeability. Values represent mean ± SEM ($n = 3$). $^*p < 0.05$ as determined by ANOVA and Bonferroni post-hoc test

concentrations of desvenlafaxine had no effect on R123 permeability across the BBB model when compared with the control (Figure 6A). Similar results for both drugs were observed in the Caco-2 cells (Figure 6B). Exposure to venlafaxine resulted in a 25% decrease in the permeability of R123 across the Caco-2 monolayer (Figure 6B). As in the BBB model, treatment with desvenlafaxine had no effect on R123 permeability in the Caco-2 cells at any of the concentrations tested (Figure 6B).

Discussion

Previously it was reported that treatment with venlafaxine in Caco-2 cells induced the expression of MDR1, the gene responsible for the

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production of P-gp [16]. The current study expanded upon these findings by examining the effect of venlafaxine and its metabolite, desvenlafaxine, on P-gp and BCRP protein expression in human brain endothelial cells. In these studies, the time frame in which drug efflux transporter expression was altered at the protein level by venlafaxine was similar to the profile observed previously for the MDR1 gene in Caco-2 cells [16]. In both cases, the venlafaxine-induced changes in expression occurred immediately, peaking in the first few hours. This rapid induction profile is characteristic of proteins mediated by the pregnane xenobiotic receptor (PXR) which, upon activation, regulates the expression of a wide range of enzymes [24] and proteins including P-gp [25] and BCRP [26]. For example, an increase in MDR1 mRNA expression was evident after just 10 min of exposure to the chemotherapeutic agent, cytarabine, in various human leukemic cell lines [27]. Another study using colonic epithelial cells observed a rapid induction of P-gp by propranolol, verapamil and rifampicin, with demonstrable increases occurring within 3 h [28], just as we did for venlafaxine and rifampicin in the HBMEC.

We are not the only group to observe such effects in the brain endothelium, either. Treatment of rat brain capillaries with the rodent PXR activator, pregnenolone-16 alpha-carbonitrile, elevated P-gp levels in as little as 1 h [29]. A rapid induction of P-gp was also reported in a rat brain vascular endothelial cell line, demonstrating significant increases in P-gp 2 h after provocation [30]. While our previous report [16] and the time course experiments in the present study indicate venlafaxine is capable of inducing rapid drug efflux transporter expression, these studies were performed using a drug concentration of 50 μM. In general, drug concentrations in this range increase the likelihood of a cytotoxic response. In our studies, no toxicity was observed in the HBMEC following exposure to either drug (50 μM) at any of the time points examined (1 to 72 h) as determined using a LDH cytotoxicity assay.

Another important aspect to consider is whether or not the concentration at which drug efflux transporter induction is observed has any clinical relevance, i.e. is the concentration required for induction achievable in the body following a normal dosing regimen. With respect to the intestine, it was previously reported that 50 μM venlafaxine significantly induced the expression of the P-gp gene, MDR1, in Caco-2 cells, a model of the human intestine [16]. In the present study, 10 μM venlafaxine significantly reduced the permeability of the drug efflux probe, R123, across Caco-2 monolayers. Using the equation reported by Liu *et al.* to calculate drug concentrations in the lumen of the intestine [31] and the average human gut volume of 1650 ml/70 kg [32], the intestinal concentration of venlafaxine at any given period of time following a 75 mg dose would range from 13 μM to 164 μM, depending on the level of absorption across the intestinal wall. The concentration of venlafaxine at which drug efflux transporter induction was observed in the Caco-2 cells ($\leqslant$50 μM) falls within this range. It should be noted that 75 mg/day is the recommended initial dose of venlafaxine; however, patients found unresponsive to this dose are administered additional doses up to 225 mg/day. This dose would obviously result in much higher concentrations of venlafaxine in the body, further increasing the potential for drug–drug interactions as a result of drug efflux transporter induction by venlafaxine.

With respect to the brain, drug–drug interactions would be of concern when the concentration of drug in the blood reaches the threshold at which efflux expression is induced. A recent study determined the pharmacokinetic parameters of venlafaxine in 14 healthy volunteers [33]. Administration of a single 75 mg dose of extended release venlafaxine resulted in peak plasma concentrations (C_{max}) ranging from 142 nM to 355 nM [33]. In our studies, 1 μM venlafaxine significantly induced the expression of both P-gp and BCRP. These effects also had functional relevance as exposure of 1 μM venlafaxine to the 'blood' side of the *in vitro* BBB model resulted in a statistically significant reduction in the 'brain' penetration of the drug efflux probe, R123. A reduced entry would be expected following induction as both P-gp and BCRP are localized within the apical or blood-facing membrane of the brain endothelium [1] and Caco-2 cells [34,35]. It should be noted that

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because venlafaxine is known to inhibit P-gp function [10], we may not fully appreciate the effect of induction on R123 permeability. Even though all the drug-containing medium is removed prior to the introduction of R123, it is likely that some residual intracellular venlafaxine will remain and antagonize P-gp function. So even though P-gp expression has been induced, a fraction of this may be inhibited by venlafaxine, partially masking the effects observed. This would be especially true for the higher concentrations of venlafaxine and may explain the diminished impact observed at 50 μM in the BBB model. Nevertheless, our studies indicate that the concentration of venlafaxine in the plasma following a 75 mg dose is just below the concentration at which drug efflux transporter induction is observed at the BBB *in vitro* (1 μM). However, as stated above, administration of doses greater than 75 mg could surpass the threshold required for drug efflux transporter induction and increase the probability of a drug–drug interaction at the BBB. In fact, it was reported that administration of a mean daily dose of 150 mg venlafaxine resulted in plasma concentrations ranging from 90 nM to 1.44 μM [36]. Elevated venlafaxine levels such as these could have a profound effect on drug efflux transporter activity at the BBB.

These results indicate that administration of venlafaxine can enhance the expression profiles of P-gp and BCRP in both the intestine and the brain. As stated before, the clinical consequence of these effects is the potential impact on other drugs administered concomitant to venlafaxine. Increased drug efflux transporter activity in the intestine can dramatically alter the oral absorption of drug, rendering it less bioavailable to the blood than predicted [14,37,38]. For drugs acting on targets within the CNS, enhanced efflux activity at the BBB has been shown to reduce entry into the brain and, as a result, diminish the therapeutic efficacy of such drugs [15]. In fact, there have been indications that these situations do transpire following venlafaxine administration. Previously we reported a study in which nine healthy volunteers were administered venlafaxine and indinavir, a known drug efflux substrate [39]. Venlafaxine coadministration resulted in a substantial decrease in the plasma concentration of indinivir when compared with indinavir dosed alone. Moreover, there was no change in the half-life of indinavir, suggesting the reduced plasma concentrations were not due to decreased metabolism [39]. Our current findings suggest the lower levels of indinavir in the plasma may have been due to enhanced drug efflux transporter activity in the intestine induced by venlafaxine. A drug interaction was also reported for venlafaxine and propafenone, a known substrate and inhibitor of P-gp [40]. Here, concomitant administration of venlafaxine and propafenone resulted in adverse effects such as visual hallucinations and psychomotor agitation. The authors surmised that these effects were initiated, in part, through their commonality with P-gp, to which they urged caution when administering venlafaxine with other drugs mediated by P-gp [40].

While our data suggest venlafaxine is an inducer of drug efflux protein expression, this does not appear to be the case for its metabolite, desvenlafaxine. Treatment with desvenlafaxine in the HBMEC did not result in a statistically significant change in the expression of either P-gp or BCRP. Additionally, desvenlafaxine treatment had no impact on the permeability of R123 across either the *in vitro* model of the BBB or Caco-2 monolayers. Together, these studies indicate desvenafaxine does not influence drug efflux transporter expression. This may not be all that unexpected considering that previous *in vitro* observations have reported desvenlafaxine is neither a substrate [11] nor inhibitor of P-gp [10], though *in vivo* studies involving P-gp knockout mice contradict the claim of the former [8,9].

While, to our knowledge, no one has specifically investigated the effect of desvenlafaxine on efflux mediated drug–drug interactions, there is indirect evidence suggesting such an interaction is unlikely. Administration of desvenlafaxine (100 mg) had minimal impact on the pharmacokinetics of a single dose of desipramine (50 mg) in a study involving healthy volunteers [41,42]. As a result, the authors suggested desvenlafaxine had little potential for drug interactions, though this assessment was made primarily in reference to metabolism through CYP2D6, of which desipramine is a substrate [41,42]. However, desipramine is also transported by P-gp [43], so the fact

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that desvenlafaxine does not substantially alter the pharmacokinetic parameters of desipramine suggests P-gp expression was not significantly altered. This is a clinical example of the lack of drug efflux transporter induction we observe with desvenlafaxine *in vitro*.

Conclusion

In the present study it was demonstrated that venlafaxine, but not desvenlafaxine, is an inducer of the drug efflux proteins, P-gp and BCRP. It was further indicated that the concentration at which this effect occurs is attainable in the body following a typical dosing regimen and could provoke drug–drug interactions with concomitant therapies. Moving forward, additional clinical data and animal studies will be necessary to better understand how these observations translate *in vivo*. Based on these preliminary results, caution should be taken as to how this antidepressant medication is currently prescribed.

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