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Lamotrigine is a substrate for OCT1 in brain endothelial cells

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ABSTRACT

The mechanisms that underpin the passage of lamotrigine at the blood–brain barrier to its site of action in the brain is poorly understood. Lamotrigine has been postulated to be delivered to its site of action in the brain favourably despite its physicochemical properties. The aim of this study was to investigate the transport of lamotrigine in an *in-vitro* model of the BBB. In this study, lamotrigine was found to have a distribution coefficient of 0 at pH 7.4 indicating that it was not highly lipophilic. Human brain endothelial cells (hCMEC/D3) were used to probe the interaction of lamotrigine with drug transporters. The uptake of lamotrigine into hCMEC/D3 cells was found to be an active process (K_m = 62 ± 14 μM; V_{max} = 385 ± 30 pmol/min/million cells). Furthermore, use of a panel of transporter inhibitors indicated that this active uptake was mediated by organic cation transporter 1 (OCT1). OCT1 mRNA and protein were shown to be expressed in hCMEC/D3 cells. KCL22 cells overexpressing OCT1 were then used to validate these findings. Lamotrigine was confirmed to be a substrate and inhibitor in OCT1-transfected KCL22 cells. A putative pharmacokinetic drug–drug interaction (DDI) between quetiapine and lamotrigine was recently reported in patients and we show here that quetiapine is a potent inhibitor of the OCT1-mediated transport of lamotrigine. This is the first time that a specific influx transporter has been shown to transport lamotrigine. The clinical implications of these findings with respect to the efficacy of lamotrigine and its potential for DDI require further investigation.

1. Introduction

The anticonvulsant drug lamotrigine is a phenyltriazine derivative (3,5-diamino-6(2,3 dichlorophenyl)-1,2,4-triazine), which blocks voltage-dependent sodium channels, thereby preventing excitatory neurotransmitter release. This drug is used in the treatment of a variety of CNS diseases including epilepsy, bipolar disorder and migraine. The efficacy of lamotrigine is dependent on both its systemic and transcellular pharmacokinetics, the latter being particularly important for drugs acting on the CNS because of the structural and functional properties of the blood–brain barrier (BBB) which are designed to protect the brain from toxins and microorganisms. However, very little is known about the transport of lamotrigine at the BBB.

Pharmacokinetic studies in rodents have shown that there are differences in lamotrigine drug concentrations between serum, cerebrospinal fluid (CSF) and brain extracellular fluid [1]. Once steady-state is reached, lamotrigine concentrations in rodent brains are significantly (2-fold) higher than serum concentrations [2,3]. An additional study accessed lamotrigine's permeability into the rodent brain by *in-situ* perfusion and compared this to its calculated lipophilicity (clog *P*) of −0.2. They suggested that lamotrigine was delivered to its site of action in the brain favourably despite its unfavourable physicochemical properties [4]. These data could imply that there is an active transport system for lamotrigine into the CNS.

Epilepsy patients show wide variation in plasma concentrations of lamotrigine, but since this is not related to clinical outcome, therapeutic drug monitoring is not indicated except during

Abbreviations: BBB, blood–brain barrier; CSF, cerebrospinal fluid; FBS, fetal bovine serum; HBSS, hanks balanced salt solution; OCT, Organic cation transporters; TEA, tetraethylammonium chloride; OCT1, organic cation transporter 1; AED, anti-epileptic drug.

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pregnancy [5,6]. In relation to adverse effects, a number of studies, including one large scale trial involving 811 patients [7], have suggested that lamotrigine plasma concentrations are linked to tolerability, whilst numerous other studies have shown no association [8–10]. The reasons for variability in treatment response are unknown. Although pharmacodynamic factors may be important, it is possible that cellular efflux and influx transporters cause variation in the pharmacokinetics and the brain exposure to lamotrigine, and therefore modulate the concentration of lamotrigine at its site(s) of action. The BBB is replete with influx transporters in addition to efflux transporters [11] and many drugs have been shown to utilise active transporters to reach the CNS at concentrations sufficient to exert their therapeutic effects (*e.g.* L-dopa [12]). It is possible that lamotrigine is also actively transported into the brain.

To test this hypothesis, we have used brain endothelial cells to identify putative transporter(s) involved in lamotrigine transport. hCMEC/D3, an immortalised human microvascular brain endothelial cell line with many of the characteristics of primary brain endothelial cells, was used for *in-vitro* studies of the BBB [13]. Organic cation transporters (OCTs) are a subfamily of influx transporters that play an important role in the distribution and excretion of cationic drugs [14]. Substrates of OCTs are generally organic cations and weak bases that are positively charged at physiological pH, but non-charged compounds have also been shown to be transported [14].

2. Materials and methods

2.1. Materials

[^{14}C]-Lamotrigine was a kind gift from Glaxo SmithKline (GSK, Stevenage, UK) with a specific activity of 52.9 mCi/mmol and [^{3}H]-lamotrigine was acquired from American Radiolabeled Chemicals (ARC, St. Louis, USA) with specific activity of 5 Ci/mmol. [^{3}H]-Topiramate, [^{3}H]-levetiracetam and [^{14}C]-valproate were acquired from ARC with specific activities of 5 Ci/mmol, 8 Ci/mmol and 55 mCi/mmol, respectively. [^{14}C]-Carbamazepine was a kind gift from Novartis (Basel, Switzerland) with specific activity of 49 mCi/mmol. [^{14}C]-Tetraethylammonium chloride (TEA) was obtained from ARC (specific activity = 55 mCi/mmol) and [^{14}C]-phenytoin was obtained from PerkinElmer (Massachusetts, USA) with specific activity of 53.1 mCi/mmol. Tariquidar was synthesised by Dr. Oliver Langer, Medical University of Vienna, Austria. Montelukast was a gift from Merck Frosst (Quebec, Canada) and quetiapine fumarate was acquired from Sequoia Research Products (Pangbourne, UK). All other drugs and chemicals, unless otherwise stated, were purchased from Sigma (Poole, Dorset, UK).

2.2. Distribution coefficient (log D, pH 7.4)

The log *D* (pH 7.4) of lamotrigine was experimentally determined as previously described [15]. In brief, the lipophilicity of a compound is defined as the distribution coefficient (log *D*) in a biphasic system. [^{3}H]-Lamotrigine was suspended in 1 mL HBSS with 25 mM HEPES at pH 7.4, and then mixed vigorously for 15 min with 1 mL 1-octanol. The layers were separated by centrifugation at 250 x *g* for 5 min at room temperature. Radioactivity was determined by scintillation counting of 500 mL aliquots from both phases. The log *D* pH 7.4 distribution coefficient was calculated as below, in which DPM represents disintegrations per minute:

$$\log D = \log\left(\frac{\mathrm{DPM_{octanol}}}{\mathrm{DPM_{buffer}}}\right)$$

2.3. Cell culture

hCMEC/D3, an immortalised human brain endothelial cell line, was maintained in EGM-2 medium (Lonza, Slough, UK) supplemented with penicillin–streptomycin (1%), hydrocortisone (1.4 μM), ascorbic acid (5 μg/mL), chemically defined lipid concentrate (1/100) (Invitrogen, Paisley, UK), HEPES (10 mM), 5% fetal calf serum (FCS) and basic fibroblast growth factor (1 ng/mL). hCMEC/D3 were passaged every 3–4 days and seeded onto collagen type I (Invitrogen) coated cell culture plasticware (Nunc, Fisher Scientific, Loughborough, UK). KCL22 wild type cells are a chronic myelocytic leukemia cell line expressing low basal levels of OCT1; previously generated stably transfected OCT1 cells expressing human OCT1 were utilised for drug transport assays [16]. In brief, the KCL22 cell line was stably transfected with pcDNA-hOCT1 plasmid (kind gift of D. Gründemann, Cologne, Germany) to give an OCT1 expression cell line and transfected with the empty vector pcDNA3 to yield mock-transfected control lines. Cells were grown in RPMI 1640 medium supplemented with 1% L-glutamine, penicillin/streptomycin, and 10% FCS. The Countess Automated Cell Counter (Invitrogen) was used to determine cell counts for plating and drug transport assays.

2.4. Cellular accumulation assay

Studies of drug accumulation utilised a tracer concentration of radiolabelled compound to give a radioactivity concentration of 0.3 μCi/mL with sufficient non-radiolabelled compound added to give a final concentration of drug from 2 μM to 300 μM in transport medium. Transport media consisted of Hanks balanced salt solution (HBSS) with 25 mM HEPES at pH 7.4 with 0.1% BSA.

hCMEC/D3 cells were seeded at a density of 7.5×10^5 per well onto collagen coated 6 well plates. Cells were cultured for 2 days. On the day of the assay, the cells were allowed to equilibrate in the transport media at 37 °C. Transport media containing the radiolabelled drug with vehicle (DMSO) or inhibitor were added to the cells and incubated at 37 °C for 30 min or for the indicated time. After incubation, three wash steps with ice-cold HBSS were performed and the washed cells were solubilised by incubation with 600 μL 10% sodium dodecyl sulphate (SDS) for 30 min. The resultant solution was mixed with 4 mL scintillation fluid, and radioactivity was counted using a scintillation-counter (1500 Tri Carb LS Counter; Packard). For the inhibition studies, the following inhibitors were used: verapamil (blocks OCT1 and ABCB1), amantadine (blocks OCT1 and OCT2), prazosin (blocks OCT1 and OCT3), naringin (blocks OATP1A2), probenecid (BCRP, MRPs and OATs including OAT3), montelukast (blocks MRP2 and influx transporters including OATP1B3 and OATP2B1), tariquidar (blocks ABCB1 and ABCG2), N-methylnicotinamide (blocks OCT2) and corticosterone (blocks OCT3).

The KCL22 mock-transfected and KCL22 OCT1-transfected cell lines were used for drug transporter assays by adding 2×10^6 cells to transport medium containing radiolabelled drug ± inhibitor, and drug uptake was measured as described above.

2.5. RNA extraction and quantitative RT-PCR

2×10^6 hCMEC/D3, KCL22 mock and KCL22 OCT1 cells were isolated, resuspended in HBSS and centrifuged at 250 × *g* for 5 min. The resultant cell pellet was resuspended in Tri reagent (1 mL) for subsequent RNA extraction as described in the manufacturer's instructions. Total human RNA samples from various whole tissues from a pool of three individuals were purchased from Ambion (Austin, USA). Following RNA extraction, reverse transcription utilising TaqMan® Reverse Transcription Reagents (Applied Biosystems, Paisley, UK) was performed. The samples were

prepared for real-time qPCR; 80 ng cDNA was combined with universal master mix, sense and antisense primers (0.4 μM each) and oligonucleotide probe (0.2 μM). Assays on demand primer and probe mixes for OCT1 (SLC22A1, Hs00427554), OCT2 (SLC22A2, Hs00533907), OCT3 (SLC22A3, Hs01009568) and GAPDH (4310884) were purchased from Applied Biosystems. GAPDH was included as a housekeeping gene. Thermal cycling conditions for all assays consisted of 15 min at 95 °C followed by 50 cycles of 15 s at 95 °C and 60 s at 60 °C. Quantification of PCR products occurred in real time and was analysed using a Bio-Rad Chromo4 real-time qPCR machine. Expression data were normalised to GAPDH expression using the comparative Ct method to determine relative expression of OCT1, OCT2 and OCT3 mRNA [17].

2.6. Cell lysate preparation and western blotting

To generate whole cell lysates, cells or human liver tissue were lysed in RIPA buffer (Thermo Scientific, Loughborough, UK) containing protease inhibitor cocktail and incubated for 30 min on ice. The lysate was subsequently centrifuged at 13,000 × *g* and protein quantification performed on the supernatant. Cell surface proteins from the cell lines were generated by a cell surface protein isolation kit (Thermo Scientific) as per manufacturer's instructions. Briefly, cells were biotinylated for 30 min at 4 °C using 20 mL 490 mM sulpho-NHS-SS-biotin solution in 1× phosphate buffered saline. The biotinylation reaction was terminated, cells washed in tris buffered saline and lysed in 1 mL lysis buffer with protease inhibitors at 4 °C for 30 min. The cell lysates were centrifuged at 13,000 × *g* for 15 min and the soluble fraction was added to NeutrAvidin agarose slurry with unbound proteins removed by washing buffer. SDS-PAGE sample buffer (200 μL) containing 50 mM DTT was added to the gel for 60 min to elute the captured proteins. The whole cell lysates and cell surface preparation were used for subsequent immunoblotting applications [18]. Primary antibodies were directed against OCT1 (1:1000, Mouse monoclonal (2C5), Abcam, Cambridge, UK), and alpha 1 sodium potassium ATPase (1:2000, mouse monoclonal (clone 464.6), Abcam).

2.7. Statistical tests and kinetics equations

All data are presented as mean ± standard deviation from three independent experiments performed in triplicate ($n = 3$). For uptake in the presence of inhibitors, an unpaired *t* test was used; a *P* value of <0.05 was taken to indicate significance. For calculation of IC_{50} values, the amount of drug uptake in pmol per million cells was plotted against $\log_{10}$ inhibitor concentration. The IC_{50} values were then determined using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, USA). Kinetics of lamotrigine uptake were determined by selecting a time point when linear transport was occurring (3 min) and then subtracting the drug accumulation in hCMEC/D3 cells in the presence of prazosin from drug accumulation in control hCMEC/D3 cells. This provides the prazosin inhibitable fraction (OCT1 mediated fraction). V_{max} was calculated by plotting the rate of drug transport by OCT1 (pmol/min/million cells) against $\log_{10}$ lamotrigine concentration (μM). GraphPad Prism 5 was used to calculate Michaelis–Menten values for lamotrigine transport in the hCMEC/D3 cells.

3. Results

3.1. Active uptake of lamotrigine by human brain endothelial cells

Lamotrigine uptake in the hCMEC/D3 cell line was significantly reduced by temperature reduction to 4 °C and by the presence of 50 μM verapamil compared to the vehicle control (Fig. 1A). This is consistent with an active process being responsible for lamotrigine uptake into the hCMEC/D3 cells.

3.2. Distribution coefficient (log D, pH 7.4) of lamotrigine

We have shown in previously published work with tyrosine kinase inhibitors that nilotinib was more lipophilic than imatinib as measured by log *D* at pH 7.4 (2.4 *vs* 0.8, respectively) and was therefore more likely to be taken up into the cells without an active transport component [15]. The lipophilicity of lamotrigine was investigated by experimentally determining the log *D* at pH 7.4 and was found to be 0.0.

3.3. Inhibitor panel identifies an OCT-like transporter as mediator of lamotrigine uptake by human brain endothelial cells

A panel of chemical inhibitors were utilised to determine which drug transporters could be mediating the lamotrigine influx process (Fig. 1B). The OCT inhibitors amantadine and prazosin caused a significant reduction of lamotrigine uptake compared to the control. Naringin (blocks OATP1A2) and probenecid (blocks OATs including OAT3) had no effect on lamotrigine uptake. Montelukast (blocks OATP1B3 and OATP2B1) at 50 μM significantly reduced the lamotrigine uptake, but at 10 μM had no significant effect. This suggests an "OCT-like" transporter is transporting lamotrigine and rules out the involvement of certain OATPs and OATs as probenecid and naringin did not affect uptake at the concentrations tested.

3.4. OCT1-dependent uptake of lamotrigine by human brain endothelial cells

To determine which organic cation transporter was mediating lamotrigine uptake in the hCMEC/D3 cell line, a number of other OCT inhibiting compounds were co-incubated with lamotrigine (Fig. 1C–G). A range of concentrations were tested to determine the IC_{50} values for the inhibitors. In order of increasing potency, the following were found to inhibit lamotrigine uptake: prazosin → verapamil → amantadine (Table 1). However, corticosterone, an inhibitor of OCT3 but not of OCT1 and OCT2 did not affect transport across a range of concentrations (Fig. 1F) indicating that OCT3 does not contribute to lamotrigine uptake. Similarly, N-methylnicotinamide, an OCT2 inhibitor, had no significant effect on lamotrigine uptake across a range of concentrations suggesting that OCT2 was unlikely to transport lamotrigine in hCMEC/D3.

3.5. Kinetics of lamotrigine transport in brain endothelial cells

A time course of lamotrigine uptake in the presence or absence of prazosin was generated to determine a linear time point for further transport rate studies (Fig. 2A). The 3-min time point for lamotrigine uptake was in the linear phase and was chosen for subsequent experiments with different concentrations of lamotrigine. The uptake rate for lamotrigine transport was found to be

Table 1
Summary of inhibitory potencies of inhibitors on lamotrigine uptake in a human brain endothelial cell line. The IC_{50} values for the inhibition of lamotrigine uptake in hCMEC/D3 cells were determined for verapamil, amantadine, prazosin, corticosterone and quetiapine.

Drug	Lamotrigine uptake IC_{50}(μM)	Comments
Verapamil	1.8 ± 1.2	Known to inhibit a number of drug transporters
Amantadine	0.9 ± 1.7	Inhibits OCT1 and OCT2 [44]
Prazosin	9.6 ± 1.3	Inhibits OCT1 and OCT3 [45]
Corticosterone	N/A	Potent inhibitor of OCT3 [45]
N-methylnicotinamide	N/A	Inhibits OCT2 [46,47]
Quetiapine	6.8 ± 1.0	Novel inhibitor of OCT1

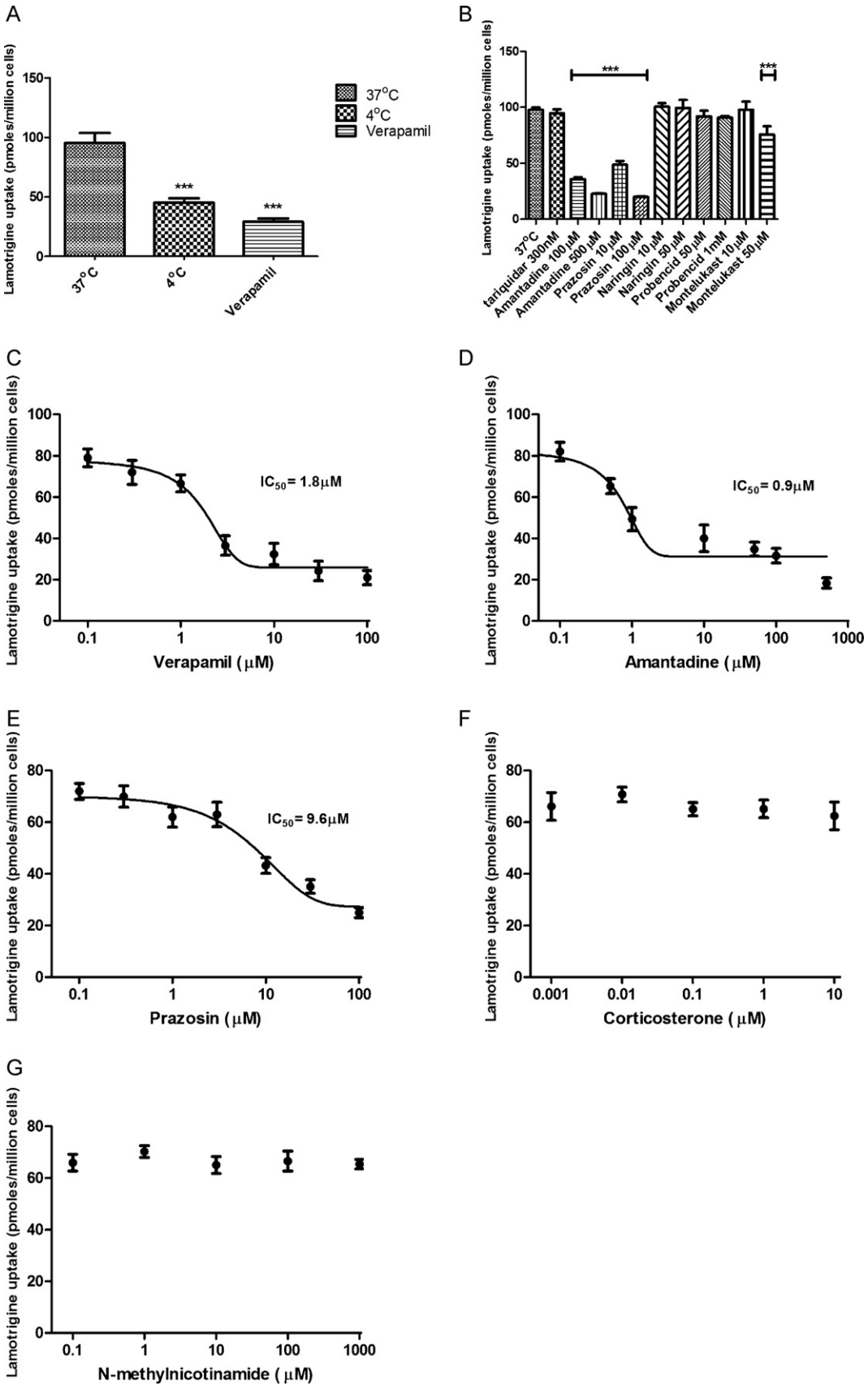

Fig. 1. Uptake of lamotrigine into human brain endothelial cells by an active transporter mediated process. (A) hCMEC/D3 cells were incubated for 30 min in transport buffer with 5 µM of [^{14}C]-lamotrigine in the presence or absence of inhibitor at 37 °C. To determine if temperature has an effect on accumulation, a study at 4 °C was performed. Data are expressed as mean ± SD (n = 3). ***Significantly different from 37 °C control ($P < .001$). (B) Uptake of lamotrigine into hCMEC/D3 cells in the presence of drug transport inhibitors. Cells were incubated for 30 min in transport buffer with 5 µM of [^{14}C]-lamotrigine ± chemical inhibitor at 37 °C. Data are expressed as mean ± SD (n = 3). ***Significantly different from control ($P < .001$). (C–G) Determination of inhibitory potencies of OCT inhibitors on lamotrigine uptake into hCMEC/D3 cells. Cells were incubated for 30 min in

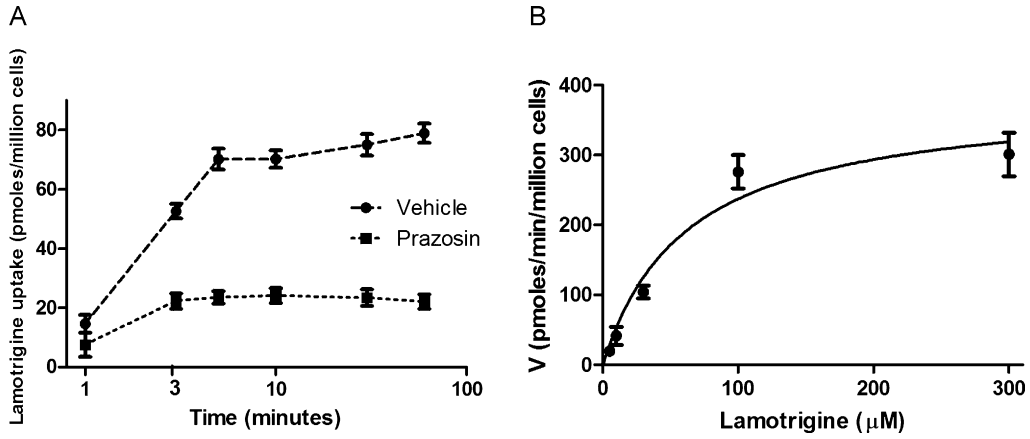

Fig. 2. Kinetics of lamotrigine uptake in human brain endothelial cells. (A) Time course of lamotrigine uptake into hCMEC/D3 cells. Cells were incubated over a time course in transport buffer with 5 μM of [^{14}C]-lamotrigine ± 100 μM prazosin at 37 °C. Lamotrigine uptake in pmol per million cells is plotted against time ($\log_{10}$ min). Data are expressed as mean ± SD (n = 3). (B) OCT1 rate of uptake of lamotrigine (prazosin inhibitable fraction) in a human brain endothelial cell line. Cells were incubated for 3 min in transport buffer with 5–300 μM of [^{14}C]-lamotrigine ± 100 μM prazosin. Lamotrigine uptake velocity in the presence of prazosin was taken away from lamotrigine uptake velocity in the presence of vehicle to give an OCT1 mediated transport rate. Log_{10} concentration of lamotrigine is plotted against the velocity of OCT1 mediated lamotrigine uptake (pmol/min/million cells). Data are expressed as mean ± SD (n = 3).

significantly different from the prazosin inhibited control over the concentration ranges of 5–300 μM. The OCT1 mediated fraction (prazosin inhibitable fraction) was plotted against the $\log_{10}$ lamotrigine concentration (Fig. 2B). The prazosin inhibitable kinetics of lamotrigine uptake were calculated with a determined K_m of 62 ± 14 μM and V_{max} of 385 ± 30 pmol/min/million cells.

3.6. Expression of OCT1 mRNA and protein in brain endothelial cells

To ascertain whether OCT1 is expressed in the hCMEC/D3 cell line, RT-qPCR was performed (Fig. 3A). KCL22 cells have low basal expression of OCT1 and following stable transfection have a ~13,000-fold increase in OCT1 mRNA relative to the KCL22 pcDNA3 mock transfected cells. The hCMEC/D3 cell line expressed 8–9-fold more OCT1than the KCL22 pcDNA3 cell line and this expression was stable between passages 35 and 58. To investigate the expression of OCT1-3 in different human tissues, RT-qPCR was performed on isolated RNA from kidney, liver, brain tissue and the hCMEC/D3 cell line (Fig. 3B). OCT1 was highly expressed in liver, with modest expression in kidney, brain and the hCMEC/D3 cell line. OCT2 mRNA was detected in the kidney, but was not detectable in brain or the hCMEC/D3 cell line (Fig. 3C). OCT3 mRNA was detected in kidney and to a lesser extent in hCMEC/D3 cells, but not in brain (Fig. 3D).

To determine if OCT1 is expressed at the protein level in hCMEC/D3 cells, immunoblotting was performed utilising a monoclonal antibody to OCT1 (Fig. 3E). Cell surface preparations and total protein lysate from a human liver were probed for OCT1 and a sodium potassium ATPase pump. Utilising the OCT1 antibody, a band at ~50 kDa was observed in the liver lysate. The OCT1 transfected KCL22 cell surface preparation showed a smeared band not observed in the control mock-transfected cells. Loading less of the cell surface preparation for the OCT1 transfected KCL22 cells showed the same smear and in hCMEC/D3 cells a band at ~70 kDa was detected. Neither of these OCT1 bands were detected in the mock-transfected KCL22 cell surface preparation suggesting specificity of the antibody. Equal protein loading for the cell surface preparations was observed from the expression of ATPase pump in each of the three cell types.

3.7. Lamotrigine is a substrate of OCT1 and can inhibit OCT1-mediated uptake

To investigate in a specific cell system whether lamotrigine was a substrate of OCT1, the KCL22 stably transfected cell line was used. As a positive control substrate, the cation TEA was found to accumulate significantly more in OCT1-transfected cells than in the mock-transfected cells, with prazosin significantly reducing this accumulation (Fig. 4A). Lamotrigine accumulation in the OCT1-transfected cells was significantly higher than in the mock-transfected cells, with prazosin inhibiting this increase (Fig. 4B), confirming that OCT1 can transport lamotrigine. Phenytoin, carbamazepine, topiramate, levetiracetam and valproate were also tested in the OCT1 transfected cell line with no significant difference in accumulation compared to the mock-transfected cell line (Fig. 4C). This suggests that these five other AEDs are not substrates of OCT1.

Next, the OCT1 mediated uptake of TEA in OCT1-transfected KCL22 cells was determined in the presence of 100 μM AED or prazosin (Fig. 5A). Only prazosin and lamotrigine at 100 μM were found to significantly decrease the uptake of TEA, suggesting that phenytoin, carbamazepine, topiramate and levetiracetam were not inhibitors of OCT1. To characterise the inhibitory potential of prazosin and lamotrigine against OCT1-mediated uptake of TEA, the IC_{50} values were determined (Fig. 5B). Prazosin was found to have an IC_{50} value of 2.7 μM ± 1.3 whilst lamotrigine had an IC_{50} value of 44.9 μM ± 1.6.

3.8. OCT1 mediated drug–drug interaction (DDI) between lamotrigine and quetiapine

A novel drug–drug interaction between quetiapine and lamotrigine has recently been described [19]. To determine if there is an interaction at the level of the OCT1 transporter, we investigated whether quetiapine was an inhibitor of OCT1. Quetiapine was found to be a potent inhibitor of OCT1-mediated transport of TEA in the transfected cells with an IC_{50} of 13.8 μM ± 2.7 (Fig. 6A). Furthermore, in the OCT1 transfected cells, an IC_{50} of 1.9 μM ± 1.0 for quetiapine was determined for inhibition

transport buffer with 5 μM of [^{14}C]-lamotrigine ± inhibitor at 37 °C. The lamotrigine uptake is plotted against a $\log_{10}$ concentration scale of inhibitor with data expressed as mean ± SD (n = 3); (C) Verapamil. (D) Amantadine. (E) Prazosin. (F) Corticosterone. (G) N-methylnicotinamide.

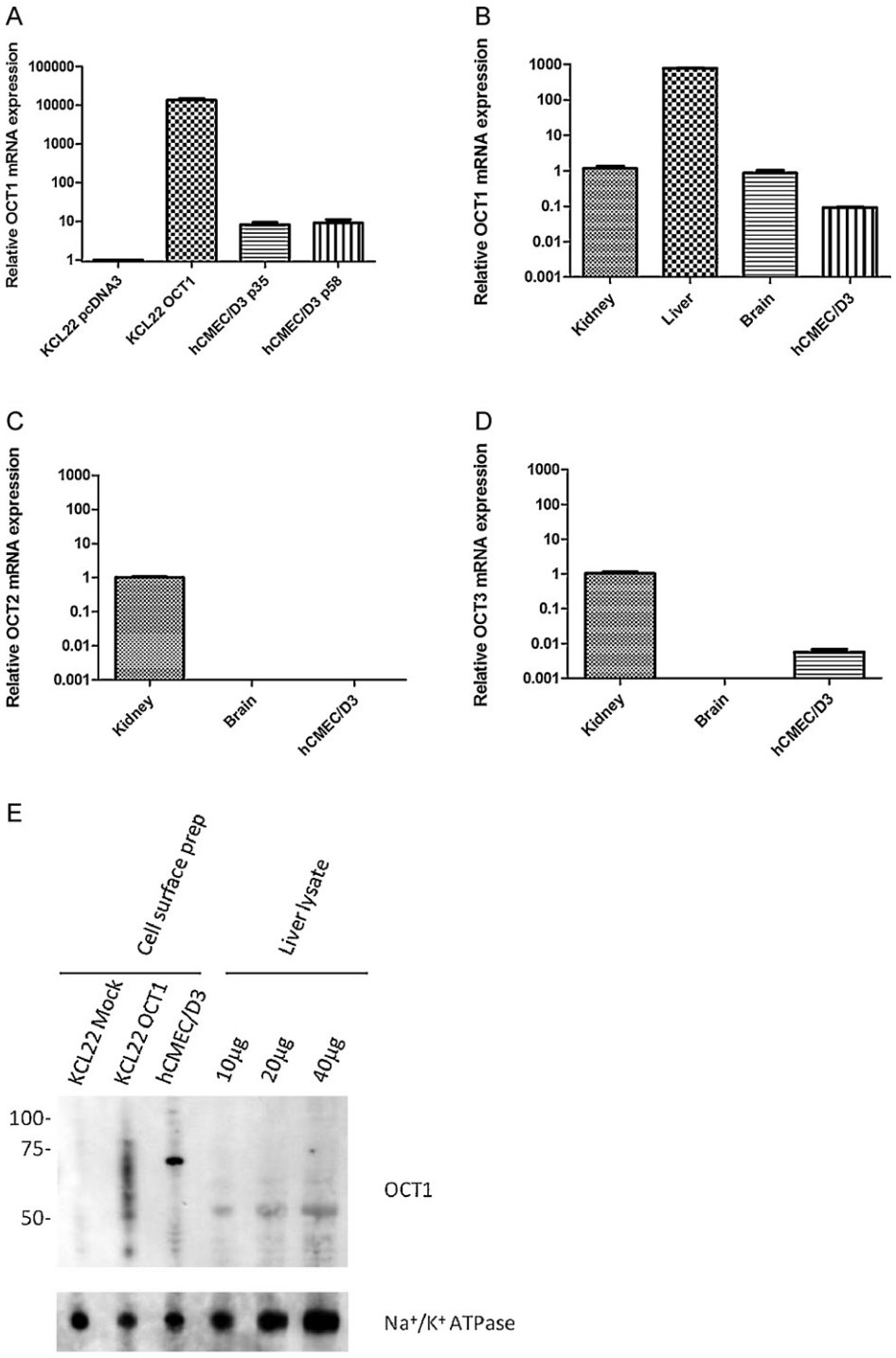

Fig. 3. Organic cation transporter expression in human brain endothelial cells and human tissues. (A) The relative mRNA expression of human OCT1 with a $\log_{10}$ scale in KCL22 pcDNA3, OCT1 transfected KCL22 and hCMEC/D3 cells. (B) Comparison of OCT1 mRNA expression with a $\log_{10}$ scale in hCMEC/D3, kidney, liver, and brain tissues (C) Determination of relative OCT2 mRNA expression with a $\log_{10}$ scale in human kidney, brain and hCMEC/D3. (D) Comparison of relative OCT3 mRNA expression with a $\log_{10}$ scale in human kidney, brain tissue and hCMEC/D3 cells. Data are expressed as mean ± SD ($n = 3$). (E) Immunoblotting for OCT1 and Na^+/K^+ ATPase pump of whole protein lysate from human liver and cell surface preparations from KCL22 pcDNA3, OCT1 transfected KCL22 cells and hCMEC/D3 cells. Representative immunoblot is shown.

of lamotrigine transport (Fig. 6B) and quetiapine was found to have an IC_{50} value of 6.8 μM ± 2.1 for inhibition of lamotrigine transport in hCMEC/D3 cells (Fig. 6C).

4. Discussion

Lamotrigine is an important anticonvulsant that is widely used in the clinic and in a recent trial was found to be clinically superior to a number of other AEDs for partial onset seizures [20]. Although lamotrigine acts within the brain, the mechanisms influencing its CNS penetration are unclear. The physicochemical properties of small molecule drugs can influence the likelihood of good BBB penetration. An optimal range of log *D* at pH 7.4 for high BBB penetration has been described to be between 2 and 4 [21]. The experimentally derived log *D* of lamotrigine at pH 7.4 was found to be 0, significantly less than this optimal range. However,

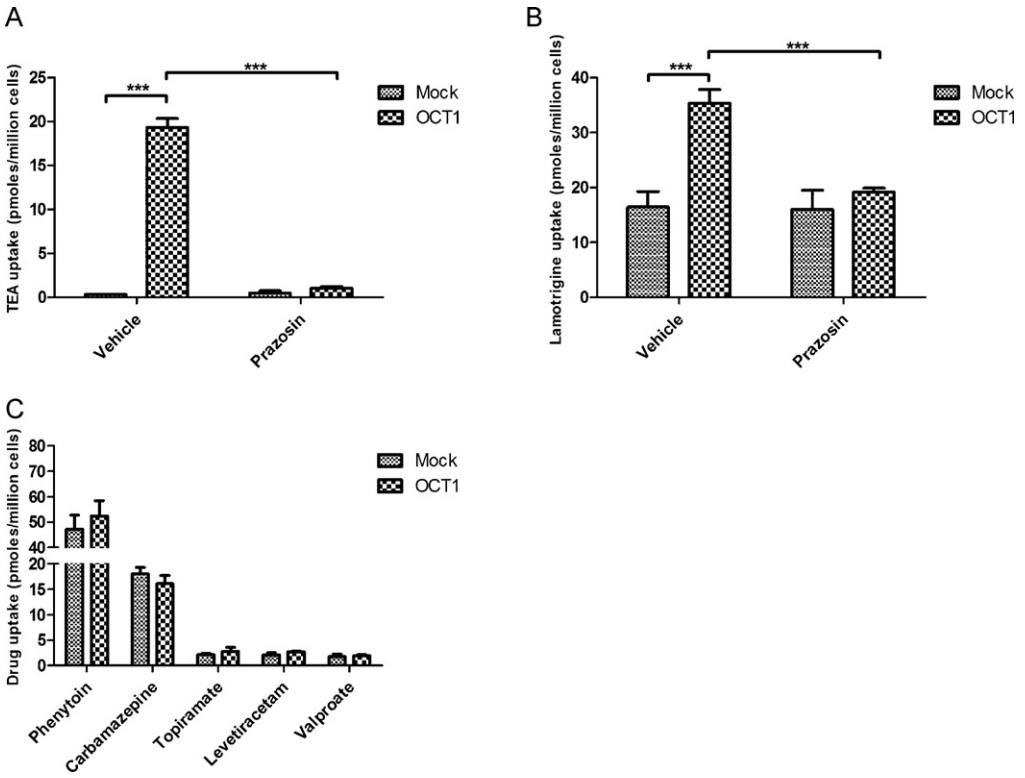

Fig. 4. Lamotrigine is a substrate of OCT1. KCL22 pcDNA3 and OCT1 transfected KCL22 cells were incubated for 30 min at 37 °C in transport buffer with; (A) 2 μM of [^{14}C]-TEA ± 100 μM prazosin. (B) 5 μM of [^{3}H]-lamotrigine ± 100 μM prazosin. (C) 5 μM of [^{14}C]-phenytoin, 5 μM of [^{14}C]-carbamazepine, 5 μM of [^{3}H]-topiramate, 5 μM of [^{3}H]-levetiracetam, and 5 μM of [^{14}C]-valproate. Data are expressed as mean ± SD (n = 3). ***Significantly different from control ($P < .001$).

lamotrigine has been shown to accumulate 2-fold more in rodent brains than in the blood, and in human brains by a factor of 2.8 [1,2,22]. Additionally, the *in-situ* perfusion of lamotrigine into the rodent brain is greater than expected taking into account its lipophilicity [4]. Therefore lamotrigine is effectively delivered to its site of action in the brain despite its unfavourable physicochemical properties, a finding consistent with the possibility that an active influx process is important. In this study, we show for the first time that an active influx process mediated by OCT1 is important in determining intracellular concentrations of lamotrigine. The influx process was characterised both in an *in-vitro* model of the BBB and in stably transfected OCT1 cells. We cannot of course exclude the possibility of other influx transporters also contributing to lamotrigine uptake, which is perhaps suggested by the high uptake in the mock-transfected KCL22 cells compared to TEA (Fig. 4).

A chemical screening approach in brain endothelial cells suggested an OCT-like transport mechanism. OCT1 is a transporter that is responsible for the carriage of cations in a facilitative process that can be in either an influx or efflux direction [14]. We used several well characterised OCT chemical inhibitors followed by a cell line over-expressing OCT1 to demonstrate its involvement in lamotrigine transport, a strategy similar to that previously used to identify imatinib as an OCT1 substrate [23,24]. The two-stage strategy is necessary since it is never possible to be sure about the specificity of chemical inhibitors for one transporter. Use of a stably transfected OCT1 cell line confirmed that lamotrigine was a substrate and an inhibitor of OCT1.

Importantly, OCT1 mRNA expression was detected in hCMEC/D3 cells and was found to be comparable to that in kidney, but far lower than liver. OCT1 protein has been described to have 3 isoforms resulting from post-translational modifications including a highly glycosylated isoform of ~70 kDa, an isoform of ~50 kDa with a low extent of glycosylation and a deglycosylated isoform of ~45 kDa [25,26]. The monoclonal OCT1 antibody used here detected endogenous expression of OCT1 in hCMEC/D3 cells, consistent with the highly glycosylated isoform, whilst in the liver, there was low glycosylation of OCT1, whilst the transfected cell line showed a smear consistent with over-expression of differentially glycosylated OCT1 isofroms. OCT1 has a well-defined role in the liver and kidney, being associated with variability in the clearance of drugs such as metformin and imatinib [27,28], and may also have a role in transport at the BBB. These findings are consistent with those of Lin et al. who showed OCT1 protein expression and activity in primary brain endothelial cells [29]. By contrast, OCT2 mRNA was not detected in the hCMEC/D3 cell line, whilst OCT3 mRNA was far lower than renal expression. The OCT1 transporter has been suggested to be expressed on the luminal membrane of brain endothelial cells and may also be responsible for the transport of the well-known neurotoxin, MPTP, into the brain [29].

It has been reported recently that patients with epilepsy receiving monotherapy have wide variability in serum concentrations of lamotrigine with a therapeutic threshold of 4–42 μM [30]. The kinetics of lamotrigine uptake determined in this study show that OCT1 transport of lamotrigine occurs at therapeutic concentrations. The system has high capacity for lamotrigine transport, as the K_m at 62 μM is above physiologically relevant concentrations. The finding that lamotrigine is a substrate of OCT1 at pharmacologically relevant concentrations provides an explanation for its higher than predicted (from physiochemical properties) penetration into the brain. It also provides a potential explanation for the lack of correlation between the serum concentrations and the therapeutic effect, as well as the CNS side effects [5], since the CNS concentrations will be more important for therapeutic and adverse effects. Brain penetration could be influenced by variation in expression and/or activity of the OCT1 transporter. It is important to note that the OCT1 gene is

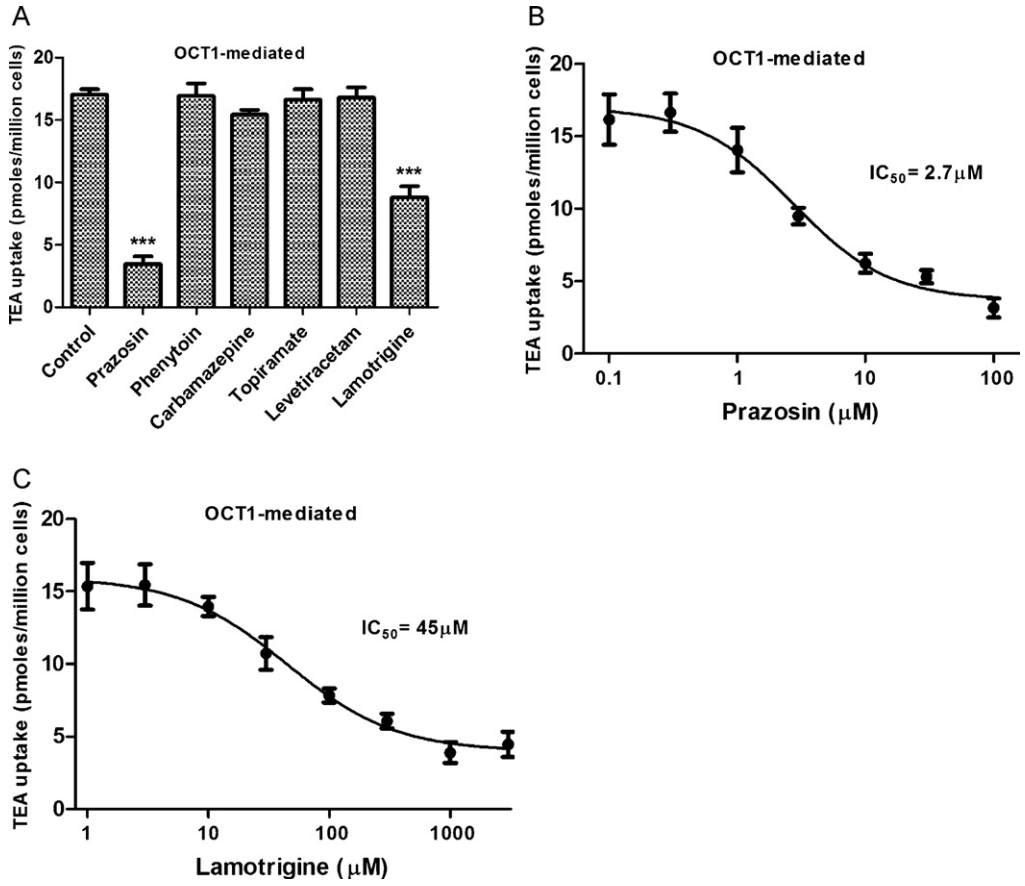

Fig. 5. Lamotrigine is an inhibitor of OCT1. (A) KCL22 cells and OCT1 transfected KCL22 cells were incubated for 30 min in transport buffer with 2 μM of [^{14}C]-TEA ± 100 μM drug. The OCT1 mediated transport is shown. (B) The 2 μM [^{14}C]-TEA uptake in OCT1 transfected KCL22 cells minus mock-transfected KCL22 is plotted against a $\log_{10}$ concentration scale of prazosin. (C) 2 μM [^{14}C]-TEA uptake in OCT1 transfected KCL22 cells minus mock-transfected KCL22 is plotted against a $\log_{10}$ concentration scale of lamotrigine. Data are expressed as mean ± SD (n = 3). ***Significantly different from 37 °C control ($P < .001$).

polymorphic with well-characterised functional variants [31,32]. With other drugs such as metformin, it is known that influx is modulated by the presence of certain SNPs; similar work with lamotrigine is now required.

There is a well-known interaction between lamotrigine and valproate which is due to inhibition of glucuronidation [33,34]. Lamotrigine metabolism is stimulated by oral contraceptives which may be related to induction of glucuronidation [35]. No pharmacokinetic DDI studies have been undertaken with a known OCT1 inhibitor and lamotrigine. However, a study with cimetidine did not show an effect on lamotrigine pharmacokinetics [36]. The lack of effect was perhaps not unexpected as cimetidine has recently been shown not to be an inhibitor of OCT1 at least when ASP+ (4-(4-(dimethylamino)styryl)-N-methylpyridinium) is used as a model substrate [37]. A DDI between quetiapine and lamotrigine was recently described using therapeutic drug monitoring [19] Specifically, lamotrigine co-administration led to significantly reduced serum concentrations of quetiapine of over 50%. This was ascribed to induction of quetiapine glucuronidation by lamotrigine, although no direct proof was provided to support this hypothesis. In this study, we found that quetiapine was a potent inhibitor in two *in-vitro* systems of the OCT1-mediated transport of lamotrigine. Patients receiving quetiapine at a dose of 250 mg 3 times a day have an average plasma concentration of between 1 μM and 1.6 μM [38] that is slightly lower than the *in-vitro* IC_{50} values for inhibition. However, the steady state C_{max} at this dose has been reported to be 2.7 μM [39] and therefore concentrations necessary to inhibit OCT1 are achieved in patients. We were not able to determine the effect of lamotrigine on the influx of quetiapine as we did not have radiolabelled quetiapine, and there are no reports in the literature regarding the transport of quetiapine. Our data would at least suggest that interaction at the level of OCT1 may also be important (at least partly) in the interaction between lamotrigine and quetiapine. With metformin, a known OCT1 substrate, interactions with drugs such as verapamil and amitriptyline occurred in a genotype-dependent manner, with the greatest effect seen with OCT1 M420del variant [40]. Further *in-vivo*/*in-vitro* studies are required to prove the involvement of OCT1 in this DDI and should also consider the possible involvement of glucuronidation. This will be important clinically as there is interest in co-administration of lamotrigine and quetiapine in treatment-resistant bipolar depression [41] and this DDI could affect the efficacy of this combination.

The occurrence of a seizure is a complex process which leads to changes in the transcription of many genes, including those coding for transporters. To this end, it is known that ABCB1 expression is increased by seizure activity [42]. In relation to OCT1, microarray analysis has shown that this was down-regulated in a rodent epilepsy model [43] but this result was not validated by RT-qPCR. The effect of seizures on OCT1 expression particularly in those patients who are refractory to treatment with lamotrigine is another important area for investigation.

In summary, this study shows that lamotrigine is transported into human brain endothelial cells by OCT1 and might explain why lamotrigine can penetrate the brain at higher concentrations than would be expected from its physicochemical properties. The clinical relevance of this finding needs further investigation – it is interesting to note that lamotrigine is less lipophilic than imatinib,

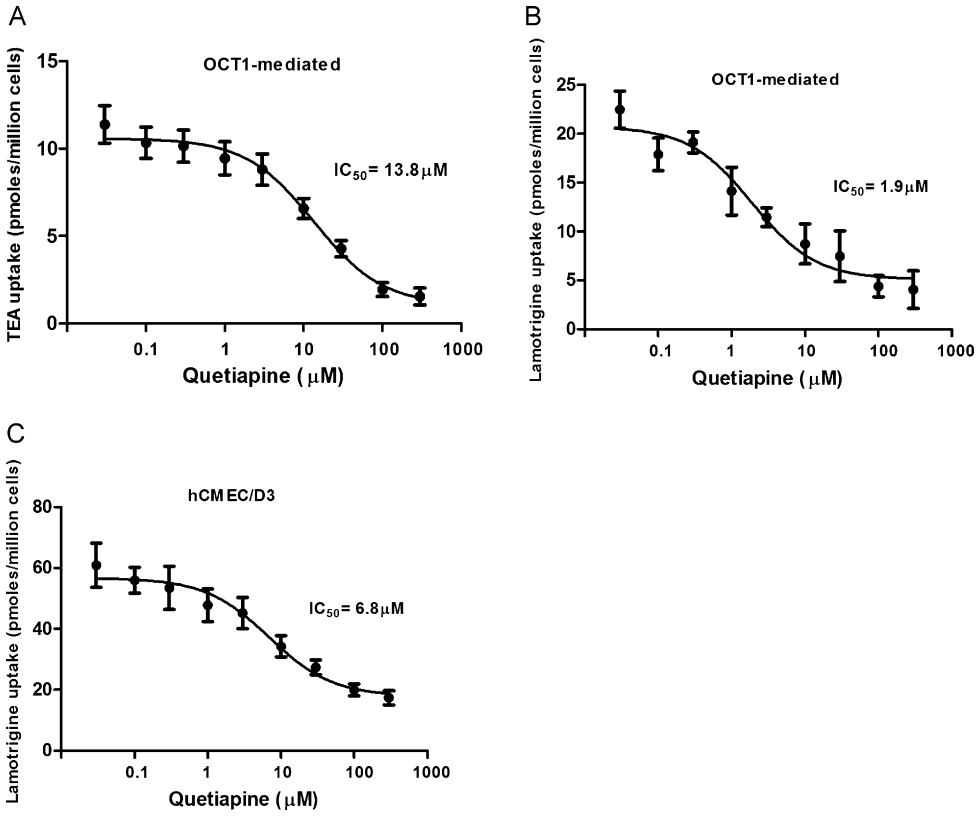

Fig. 6. OCT1-mediated drug–drug interaction between quetiapine and lamotrigine. (A) 2 μM [^{14}C]-TEA uptake in OCT1 transfected KCL22 cells minus mock-transfected KCL22 is plotted against a $\log_{10}$ concentration scale of quetiapine. (B) 5 μM of [^{3}H]-lamotrigine uptake in OCT1 transfected KCL22 cells minus mock-transfected KCL22 is plotted against a $\log_{10}$ concentration scale of quetiapine. (C) 5 μM of [^{3}H]-lamotrigine uptake in hCMEC/D3 cells is plotted against a $\log_{10}$ concentration scale of quetiapine. Data are expressed as mean ± SD (n = 3).

which has been characterised to have an active uptake process that can affect clinical outcomes [24]. Although our finding does not rule out the possibility that other transporters are also important in determining the intracellular concentrations of lamotrigine, the data are important because this is the first demonstration of influx transporters having a role for this important anticonvulsants in an *in-vitro* model of the BBB.

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