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^{18}F-FCWAY, a serotonin 1A receptor radioligand, is a substrate for efflux transport at the human blood-brain barrier

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Abstract

Efflux transporters at the blood-brain barrier can decrease the entry of drugs and increase the removal of those molecules able to bypass the transporter. We previously hypothesized that ^{18}F-FCWAY, a radioligand for the serotonin 5-HT$_{1A}$ receptor, is a weak substrate for permeability glycoprotein (P-gp) based on its very early peak and rapid washout from human brain. To determine whether ^{18}F-FCWAY is a substrate for P-gp, breast cancer resistance protein (BCRP) and multidrug resistance protein (MRP1) — the three most prevalent efflux transporters at the blood-brain barrier — we performed three sets of experiments. *In vitro*, we conducted fluorescence-activated cell sorting (FACS) flow cytometry studies in cells over-expressing P-gp, BCRP and MRP1 treated with inhibitors specific to each transporter and with FCWAY. *Ex vivo*, we measured ^{18}F-FCWAY concentration in plasma and brain homogenate of transporter knockout mice using γ-counter and radio-HPLC. *In vivo*, we conducted positron emission tomography (PET) studies to assess changes in humans who received ^{18}F-FCWAY during an infusion of tariquidar (2 – 4 mg/kg iv), a potent and selective P-gp inhibitor. *In vitro* studies showed that FCWAY allowed fluorescent substrates to get into the cell by competitive inhibition of all three

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transporters at the cell membrane. *Ex vivo* measurements in knockout mice indicate that ^{18}F-FCWAY is a substrate only for P-gp and not BCRP. *In vivo*, tariquidar increased ^{18}F-FCWAY brain uptake in seven of eight subjects by 60–100% compared to each person's baseline. Tariquidar did not increase brain uptake via some peripheral mechanism, given that it did not significantly alter concentrations in plasma of the parent radioligand ^{18}F-FCWAY or its brain-penetrant radiometabolite ^{18}F-FC. These results show that ^{18}F-FCWAY is a weak substrate for efflux transport at the blood-brain barrier; some radioligand can enter brain, but its removal is hastened by P-gp. Although ^{18}F-FCWAY is not ideal for measuring 5-HT$_{1A}$ receptors, it demonstrates that weak substrate radioligands can be useful for measuring both increased and decreased function of efflux transporters, which is not possible with currently available radioligands such as ^{11}C-loperamide and ^{11}C-verapamil that are avid substrates for transporters.

Keywords

PET; efflux transporter; P-gp; blood brain barrier; 5-HT$_{1A}$

Introduction

ATP-binding cassette (ABC) efflux transporters at the blood-brain barrier can decrease the entry of drugs and increase the removal of those molecules able to bypass the transporter. For therapeutic drugs, the decreased uptake can cause drug resistance, as likely occurs in drug-resistant epilepsy (for a review, see (Loscher and Sills, 2007)). For radioligands, the decreased uptake can confound the interpretation of brain imaging studies. That is, it is unclear whether decreased radioligand uptake reflects decreased density of the radioligand's target or increased transport out of brain. Indeed, this issue may confound prior findings of decreased uptake of the 5-HT$_{1A}$ receptor radioligand ^{18}F-FCWAY (Lang et al., 1999) in the epileptogenic focus of patients with temporal lobe epilepsy (Theodore et al., 2007). The authors interpreted the results to mean that 5-HT$_{1A}$ receptors were decreased in the temporal lobe of patients, but the findings could also reflect increased removal of the radioligand from brain. In fact, other 5-HT$_{1A}$ radioligands with a structure similar to that of FCWAY (Liow et al., 2007; Passchier et al., 2000) have been reported to be substrates for permeability-glycoprotein (P-gp), one of the two most prevalent efflux transporters at the blood-brain barrier. In addition, most patients in that study were drug-resistant. Interestingly, *in vitro* measurement of P-gp in surgically resected brain suggests that P-gp overexpression causes this resistance (Sisodiya et al., 2002), a finding also supported by *in vivo* PET imaging using a radiolabeled P-gp substrate (Feldmann et al., 2013). As an example of another characteristic of radioligand uptake mediated by efflux transporter, a recent study found that brain uptake of ^{18}F-Mefway in rats pretreated with tariquidar increased by 1.6-fold, but binding potential (BP_{ND}) did not change much after the treatment (Choi et al., 2016).

We previously hypothesized that ^{18}F-FCWAY in humans is a weak substrate for P-gp based on its very early peak in brain (within one to three minutes of injection) followed by rapid washout (Ryu et al., 2007). This time-activity curve is consistent with the existence of some contravening force (i.e., efflux transport) that increases removal of radioligand from brain. Furthermore, administration of disulfiram, which was used to inhibit defluorination of ^{18}F-

FCWAY, created the far more common time-activity curve for a receptor radioligand: peak uptake at five to seven minutes, followed by slow washout. ^{18}F-FCWAY is certainly not an avid substrate for P-gp, because if this were so no radioligand would have been able to enter brain. Nevertheless, it may be a weak substrate for efflux transport at the blood-brain barrier, which would allow some radioligand to enter brain; P-gp would then hasten radioligand removal. Disulfiram, which has many non-specific actions on the transporters, may have acted as a P-gp inhibitor (Nagendra et al., 1993).

To determine whether ^{18}F-FCWAY is a substrate for P-gp in humans, we examined the effect of tariquidar, a potent and selective P-gp inhibitor, using human cells transcribed with transporter expressed gene, transporter knockout mice, and *in vivo* human PET imaging following ^{18}F-FCWAY injection. Because many substrates can be transported by either P-gp, breast cancer resistance protein (BCRP), or multidrug resistant protein (MRP1) — the three most prevalent ABC transporters at the human blood-brain barrier — we also examined the effect of BCRP and MRP1 in human cells culture and transgenic mice.

Methods

In vitro measurement of FCWAY as substrate for efflux transport

Methods for the experimental design were adapted from (Kannan et al., 2010). Briefly, because substrates at high concentrations can competitively inhibit transporter function (Ambudkar et al., 1999), we assessed the ability of FCWAY to inhibit the function of the three most prevalent ABC transporters at the blood-brain barrier: P-gp, BCRP, and MRP1. We tested the ability of FCWAY (20 & 50 μM) to inhibit the efflux of fluorescent substrates from human cancer cells expressing P-gp (*ABCB1*, rhodamine-123), BCRP (*ABCG2*, mitoxantrone), or MRP1 (*ABCC1*, calcein-AM) using fluorescence-activated cell sorting (FACS) flow cytometry. Four conditions for each transporter were tested (Figure 1): 1) untreated parental cells (negative control); 2) transporter-expressing cells treated by transporter-specific inhibitor; 3) transporter-expressing cells treated by substrate (FCWAY); and 4) untreated transporter-expressing cells (positive control). Fluorescence intensity (cellular uptake of fluorescent substrate) was recorded for a total of 10,000 cells using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). FACS data were analyzed using FlowJo software (Tree Star, Inc., Ashland, OR).

Ex vivo mice study

Four types of mice—wild type, P-gp knockout, BCRP knockout, and P-gp/BCRP knockout (Taconic Farm, Germantown, NY)—were used (three in each group (25.3 ± 1.2 g)). All animals were studied in accordance with the *Guide for the Care and Use of Laboratory Animals* and were approved by the National Institute of Mental Health Animal Care and Use Committee.

After inhalation of anesthesia with 1.5% isoflurane in oxygen, each mouse was injected intravenously through its cannulated tail vein with ^{18}F-FCWAY (166 ± 49 μCi, specific activity: 826 ± 205 mCi/μmol) and was allowed a 30-min survival period. At the end of 30 min, a blood sample was collected through cardiac puncture followed by brain extraction.

The injection took place sequentially every 10 min to allow time for drawing blood and brain excision. Whole blood samples and brain homogenates were analyzed by radio-HPLC using reversed-phase chromatography (X-terra, Waters Corp., Milford, MA) and quantification of radioactivity by γ-counter (model 1480 Wizard; Perkin-Elmer). To correct for the input functions, ratios of the parent radioligand ^{18}F-FCWAY in brain tissues to those in their antilogous plasmas were obtained and compared between the four groups of mice.

PET imaging

Subjects—Eight healthy subjects (six men and two women; 26 ± 3 y; 72 ± 10 kg) each underwent two brains scan with ^{18}F-FCWAY. All subjects were free of medical and neuropsychiatric diseases, as determined by medical history, physical examination, electrocardiogram, and laboratory blood and urine tests (including blood count and serum chemistry). All subjects had a negative drug screen at the time of examination. Vital signs were monitored before ligand injection, and then during and after completion of the scan. Urinalysis and blood lab tests were repeated within a few hours of completion of the PET scan. All subjects were followed up 24 hours after their PET scan. The study was approved by the Institutional Review Board of the National Institute of Mental Health, and all subjects signed a consent form to participate in the study.

Image acquisition—For each subject, a baseline scan was acquired first, followed by a 'tariquidar scan' on a later date (> two weeks) both with full arterial blood sampling. After a bolus injection of ^{18}F-FCWAY (376 ± 8 MBq), a PET scan was acquired on a GE advance scanner for two hours with increasing frame duration of 30 seconds to five minutes. For the tariquidar scan, the potent and selective P-gp inhibitor tariquidar was administered at 2 mg/kg (two subjects) or 4 mg/kg (six subjects) via constant infusion during the PET scan. Infusion began 30 minutes before radioligand injection and continued at a rate of 2 mg/kg/h, ending 30 minutes and 90 minutes after radioligand injection, respectively. The infusion rate was kept constant to avoid adverse effects (Kreisl et al., 2010). Images were reconstructed using 3D filtered backprojection with scatter, attenuation, and frame-based motion correction.

Plasma measurements—Arterial blood samples (1.5 – 6.0 mL each) were drawn from the radial artery from an initial 15-second interval to 15-minute interval towards the end of the PET scan. Plasma parent concentration and whole blood radioactivity were measured. Two radiometabolites—the fluoride ion ($^{18}F^{-}$) and the acid (^{18}F-FC)—were separated by high performance liquid chromatography (HPLC) and measured (Ma et al., 2003). Plasma free fraction was also measured (Gandelman et al., 1994).

Image preprocessing—A high resolution T1-weighted MRI brain image was acquired for all subjects for anatomical reference. Dynamic PET images were spatially normalized to Montreal Neurological Institute (MNI) space using Statistical Parametric Mapping (SPM8, Wellcome Department of Cognitive Neurology, London, UK). Volumes of interest from the Automated Anatomical Labeling (AAL) template (Tzourio-Mazoyer et al., 2002) were combined into 12 regions and applied to the spatially normalized image to obtain time activity curves. To avoid skull contamination due to defluorination of the radioligand, only

six centrally located brain regions were selected for the subsequent compartmental analysis: hippocampus, amygdala, insula, cingulate cortex, striatum, and cerebellum.

Quantification of ^{18}F-FCWAY uptake—In order to account for the ^{18}F-FC radiometabolite entering the brain, we used a three-tissue compartmental model (two compartments for ^{18}F-FCWAY and one compartment for ^{18}F-FC (Carson et al., 2000)) to calculate distribution volume (V_T). All plasma parent and whole-blood curves were fitted by a tri-exponential function and used as input. The blood ^{18}F-FC curve was fitted by a bi-exponential function and used as input for the metabolite. Rate constants for the metabolite compartment (K_{1m} and k_{2m}) were fixed based on prior measurements in monkey by injection of ^{18}F-FC directly (Carson et al., 2003). Rate constants for the parent (K_{1p}, k_{2p}, k_3, k_4) were estimated together with V_T. The final fitting of brain data were weighted by the frame-wise noise-equivalent counts proportional to the standard deviation of each frame.

Results

In vitro and ex vivo measurements

Using human cancer cells grown to overexpress P-gp, BCRP, or MRP1, we found that FCWAY is a weak substrate for all three transporters (Table 1). This *in vitro* assay indirectly assesses transport based on the ability of the ligand at high concentrations to inhibit the efflux of fluorescently labeled substrates of these transporters via “substrate inhibition” (Kannan et al., 2010). For direct assessment of ^{18}F-FCWAY as a substrate in transporter knockout mice, we found that, in comparison to wild type mice, the ^{18}F-FCWAY uptake was increased in P-gp KO mice but not in BCRP KO mice. Furthermore the combined knockout of P-gp and BCRP had no significantly greater effect that P-gp knockout alone (Figure 2). These *ex vivo* data show ^{18}F-FCWAY is a substrate for P-gp and not BCRP at the blood-brain barrier in mice.

Tariquidar administration to maximize plasma concentrations

Tariquidar was administered as a constant infusion over one or two hours, for a total dose of 2 or 4 mg/kg (Figure 3). As we previously reported (Kreisl et al., 2015), the plasma concentration of tariquidar rapidly declined at the end of the infusion (Figure 3), presumably because its initial rate of distribution to organs of the body is rate-limiting. We used this rate-limiting effect and injected ^{18}F-FCWAY during the infusion of tariquidar, when plasma concentrations were high and inhibition of P-gp would be maximal. Specifically, tariquidar infusion began 30 minutes before injection of the radioligand (t=0, Figure 3). Doubling the total dose from 2 to 4 mg/kg but maintaining a constant rate of infusion (2 mg/kg/h) allowed us to maintain plasma tariquidar concentrations at their highest levels for 1.5 hours rather than only 30 minutes.

PET imaging

After ^{18}F-FCWAY injection, brain radioactivity uptake quickly peaked around 3 SUV and was followed by moderate washout (Figure 4). With tariquidar treatment, all subjects except one showed a moderate signal increase in all brain regions and a delayed peak (Figure 4). For the six regions analyzed, there was a statistically significant increase in mean V_T of 60–

100% (hippocampus: $p < 0.005$; insula $p < 0.006$; cingulate $p < 0.004$; striatum $p < 0.006$; amygdala $p < 0.007$; cerebellum: $p < 0.014$) (Figure 5). Because the selected regions are centrally located in the brain, contamination from skull radioactivity uptake due to defluorination was negligible.

Brain ^{18}F-FCWAY uptake increased after tariquidar infusion. However, neither the parent radioligand concentration (Figure 6A) nor the concentration of ^{18}F-fluoride (Figure 6B) were affected. Peak uptake of the other radiometabolite (^{18}F-FC) in plasma increased slightly after tariquidar treatment, as did the estimated ^{18}F-FC concentration in brain (Figures 6C & 6D). Nevertheless, the concentration of ^{18}F-FC in brain was relatively small (~ 5%) compared to the total radioactivity and was accounted for in the compartmental modeling. Plasma free fraction was elevated after tariquidar in six of the eight subjects, with a mean increase of 20%; this increase was not statistically significant (paired t-test, $p = 0.06$). Finally, time-activity curves from the skull and pituitary gland—both of which lie outside the blood-brain barrier—did not change before and after tariquidar treatment (Figure 7).

Discussion

Here, we found that ^{18}F-FCWAY is a substrate for P-gp in humans. Although *in vitro* studies using human cancer cells overexpressing the transporters found that FCWAY is a weak substrate for all three ABC transporters, the assay is indirect based on competitive inhibition. *Ex vivo* studies in transporter knockout mice, which used a stronger and more direct assay, indicated that only P-gp was involved in mice. *In vivo*, the selective P-gp inhibitor tariquidar increased brain uptake of ^{18}F-FCWAY in seven of eight subjects by 60–100% compared to each person's baseline. Tariquidar did not increase brain uptake via some peripheral mechanism, as it did not significantly change the concentrations in plasma of the parent radioligand ^{18}F-FCWAY or its brain-penetrant radiometabolite ^{18}F-FC; tariquidar also did not change the plasma protein binding of ^{18}F-FCWAY. Although we could test ^{18}F-FCWAY as a substrate for BCRP or MRP1 *in vitro*, we could not do so *in vivo*, as a BCRP- or MRP1-specific inhibitor is not available for use in humans. Taken together, the results show that ^{18}F-FCWAY is a weak substrate for efflux transport at the blood-brain barrier, which allows some radioligand to enter brain; P-gp then hastens removal of the radioligand.

To our knowledge, the terms "avid" and "weak" do not have a consensus definition in the field. Here, we have used "avid" to refer to substrates that have no or negligible uptake into brain. At the opposite end of the spectrum are drugs whose brain uptake is unaffected by efflux transporters. By this terminology, "weak" can refer to any substrate in between the extremes, but we focus on those that have "moderate" uptake in hopes that increased or decreased efflux transporter function would be reflected in the opposite action on brain uptake of radiolabeled substrates. Defined in this manner, many anti-epileptic drugs would be considered weak substrates because—although they enter brain adequately at initiation of treatment—increased expression of efflux transporters may subsequently lead to drug resistance (Bauer et al., 2008).

Prior efforts to image P-gp function tended to focus on radioligands such as ^{11}C-verapamil and ^{11}C-desmethyl-loperamide (^{11}C-dLop) (Kreisl et al., 2010; Liow et al., 2007), which are selective and avid substrates for P-gp. Although selectivity is useful in interpreting the results, their high avidity prevents measurement of increased function of P-gp, as is thought to occur in some conditions of drug resistance. That is, P-gp almost completely blocks uptake of these avid substrates under normal conditions; because of this 'floor effect' abnormal conditions of increased P-gp function cannot be detected by the radioligand itself as it would require even lower signal. Instead, researchers have used the avid substrate ^{11}C-verapamil in combination with tariquidar to create measureable uptake in brain. Using this technique, Feldmann and colleagues (Feldmann et al., 2013) found that patients with drug-resistant epilepsy, in comparison to control subjects, had a blunted response to tariquidar, consistent with drug resistance being caused by overexpression of P-gp. However, the combination of pharmacological inhibition with PET imaging added significant variance to the results, likely due to inter-subject differences in the pharmacokinetics and pharmacodynamics of the inhibitor.

Drug resistance might be more directly assessed using radiolabeled weak substrates, as they should have measureable uptake at baseline, and co-administration of the P-gp inhibitor would be unnecessary. In fact, prior studies using ^{18}F-FCWAY found decreased uptake in the temporal lobe of patients with drug-resistant epilepsy (Theodore et al., 2007), which may have been caused in part by increased expression of P-gp. As a further example, Mansor and colleagues recently reported the synthesis of ^{11}C-phenytoin, an anti-epileptic drug that often leads to resistance (Mansor et al., 2015). Although phenytoin is a substrate for both P-gp and BCRP, its brain uptake would surely be a direct measure of resistance at least to this medication and perhaps others that are associated with drug resistance (Nakanishi et al., 2013).

A radiolabeled weak substrate not only overcomes the "floor effect" of avid substrates that have no brain uptake at baseline, but also the "spare capacity" shown by many efflux transporters at the blood-brain barrier. P-gp and BCRP are so highly concentrated in the endothelium of only the capillary segments of the vascular system that a small percentage (probably <10%) can completely block the uptake of avid substrates (Kannan et al., 2013). This spare capacity means that a high percentage of transporters must be blocked to increase the uptake of an avid substrate. Thus, radiolabeled avid substrates are not only incapable of measuring pathological conditions of increased P-gp function (because of the floor effect) but are also relatively insensitive to conditions of decreased P-gp function, as the decrease needs to be more than 90% before it is detected as increased uptake of the avid substrate. In summary, although weak substrates tend to be (or may always be) non-selective for efflux transporters, they are expected to be sensitive to both pathological increases and decreases of transporter function, whether the function is altered in only one or multiple transporters.

One limitation of this study is that the effect of tariquidar on ^{18}F-FCWAY must be distinguished from any potential effect it may have on two problematic metabolites generated by this radioligand: ^{18}F-fluoride ion that accumulates in skull and ^{18}F-FC that enters brain. With regard to ^{18}F-fluoride ion, we minimized and perhaps eliminated spill in from the skull by examining regions (e.g., striatum and hippocampus) in the center of brain.

In addition, tariquidar had no significant effect on uptake of radioactivity in skull. In contrast to humans, mice and rats demonstrate much greater defluorination and thus no region in its small brain would avoid the spill-in contamination of radioactivity from skull (Tipre et al., 2006). For this reason, we did not image the transgenic mice; instead, we extracted radioactivity from brain and measured the parent radioligand separated from radiometabolites. With regard to the radiometabolite ^{18}F-FC, the model accounted for its entry into brain. Furthermore, the estimated concentration in brain was only about 2–3% percent of that of the parent radioligand during most of the scan (Fig 6D). Thus, the evidence suggests that the presence of this brain-penetrant radiometabolite only minimally detracts from the conclusion that ^{18}F-FCWAY is a substrate for efflux transporters at the human blood-brain barrier. Radioligands other than ^{18}F-FCWAY are needed to assess the function of efflux transporters uncontaminated by specific receptor binding. In this regard, ^{11}C-quinidine is a possibility, although its brain signal may be contaminated with radiometabolites, which themselves may be substrates for efflux transporters (Syvanen et al., 2013).

In summary, we found that ^{18}F-FCWAY is a weak substrate for P-gp based on *in vitro* studies using human cancer cells, *ex vivo* studies using transgenic mice, and *in vivo* studies in humans scanned at baseline and during the intravenous administration of tariquidar, a selective inhibitor of P-gp. By definition, a weak substrate is one that has some brain uptake at baseline and is, therefore, sensitive to pathological conditions of increased transporter function, as likely occurs in some conditions of drug resistance. Therefore, caution should be used in interpreting the brain uptake of radioligands even if they are only weak substrates for efflux transporters, especially if used in disorders that are associated with drug resistance.

Conclusion

Our studies confirmed that ^{18}F-FCWAY is a weak substrate for P-gp. In humans, mean brain uptake increased 60–100% after *in vivo* inhibition of P-gp with some inter-subject variability. As a PET radioligand for imaging P-gp function, a weak substrate such as ^{18}F-FCWAY is more advantageous because it is sensitive to both increases and decreases in transporter function. However, interpretation of the regional variation of P-gp function is confounded by ^{18}F-FCWAY's affinity for 5-HT$_{1A}$ receptors, limiting its utility.

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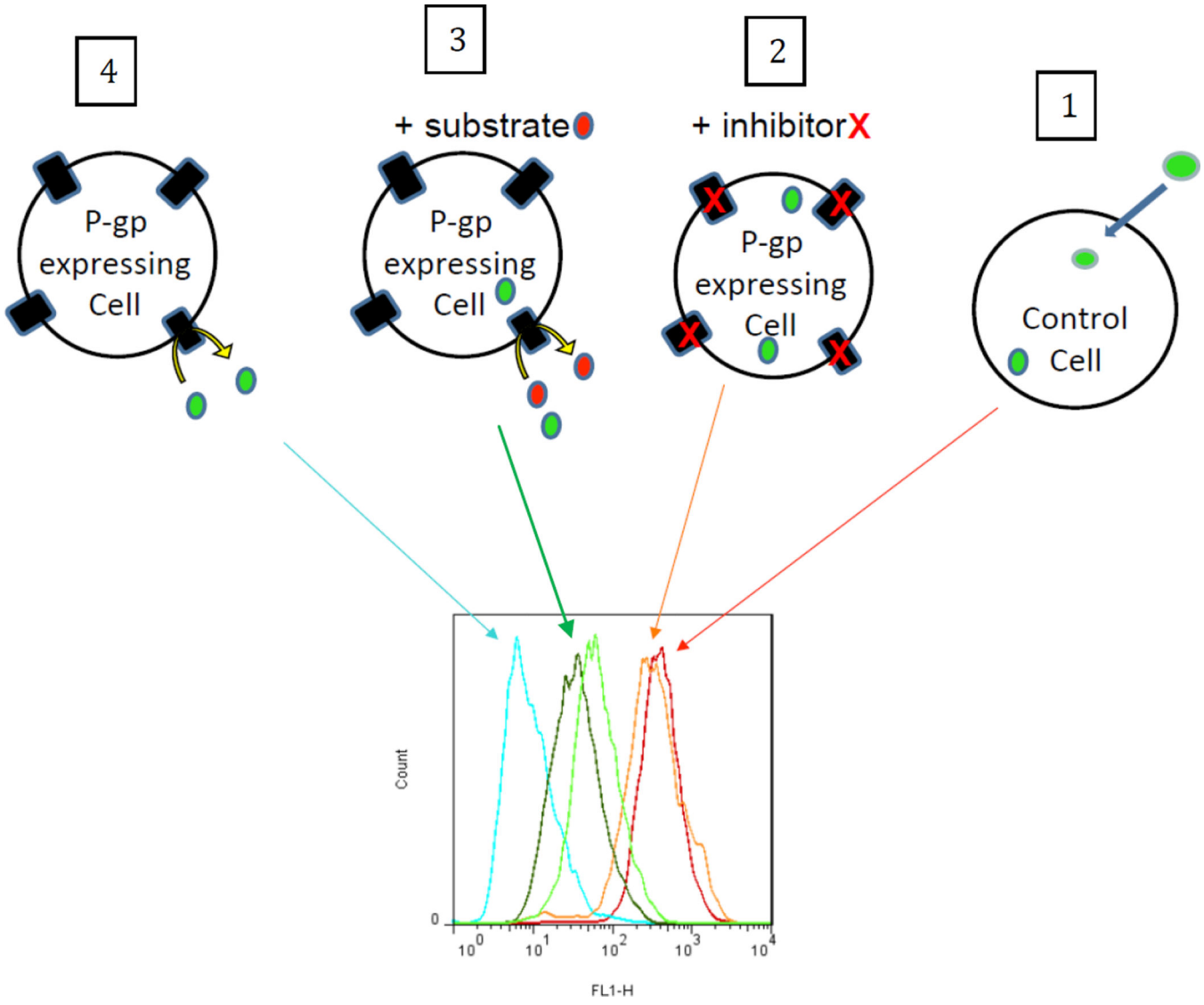

Figure 1.

Schematic illustration of fluorescence-activated cell sorting (FACS) flow cytometry with the four conditions to measure FCWAY as a substrate for each of the three ABC efflux transporters: 1) untreated parental cells (negative control); 2) transporter-expressing cells treated by transporter-specific inhibitor; 3) transporter-expressing cells treated by substrate (FCWAY); and 4) untreated transporter-expressing cells (positive control). Relative fluorescence units (intensity) is shown on the x-axis. Cell count for 10,000 events is shown on the y-axis.

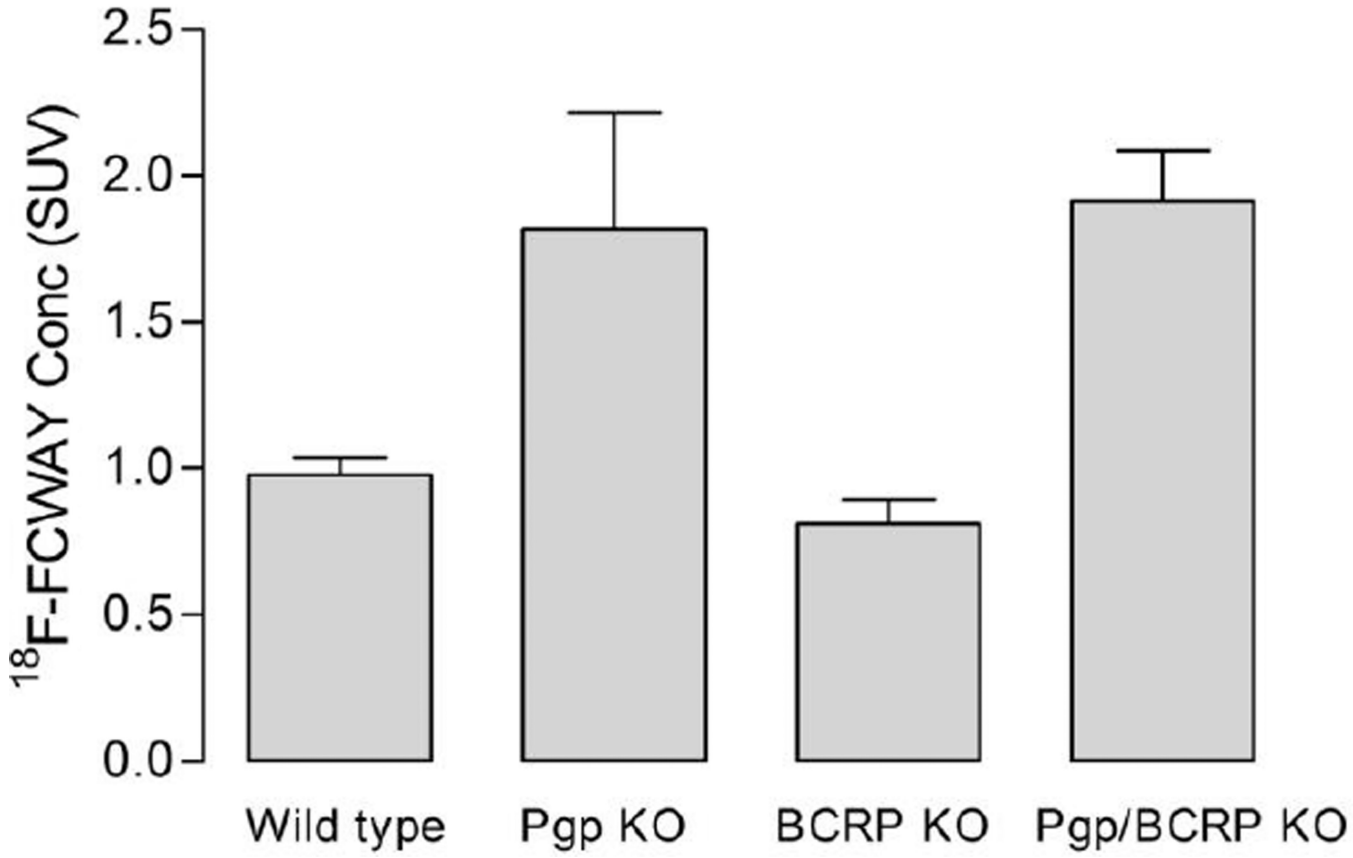

Figure 2.

Brain to plasma ratio of ^{18}F-FCWAY concentration in four stains of transgenic mice: wild type, P-gp KO, BCRP KO, and combined P-gp and BCRP KO (all n=3). RadioHPLC was used to measure the parent radioligand ^{18}F-FCWAY separated from radiometabolites. Error bars represent standard error of the mean (SEM).

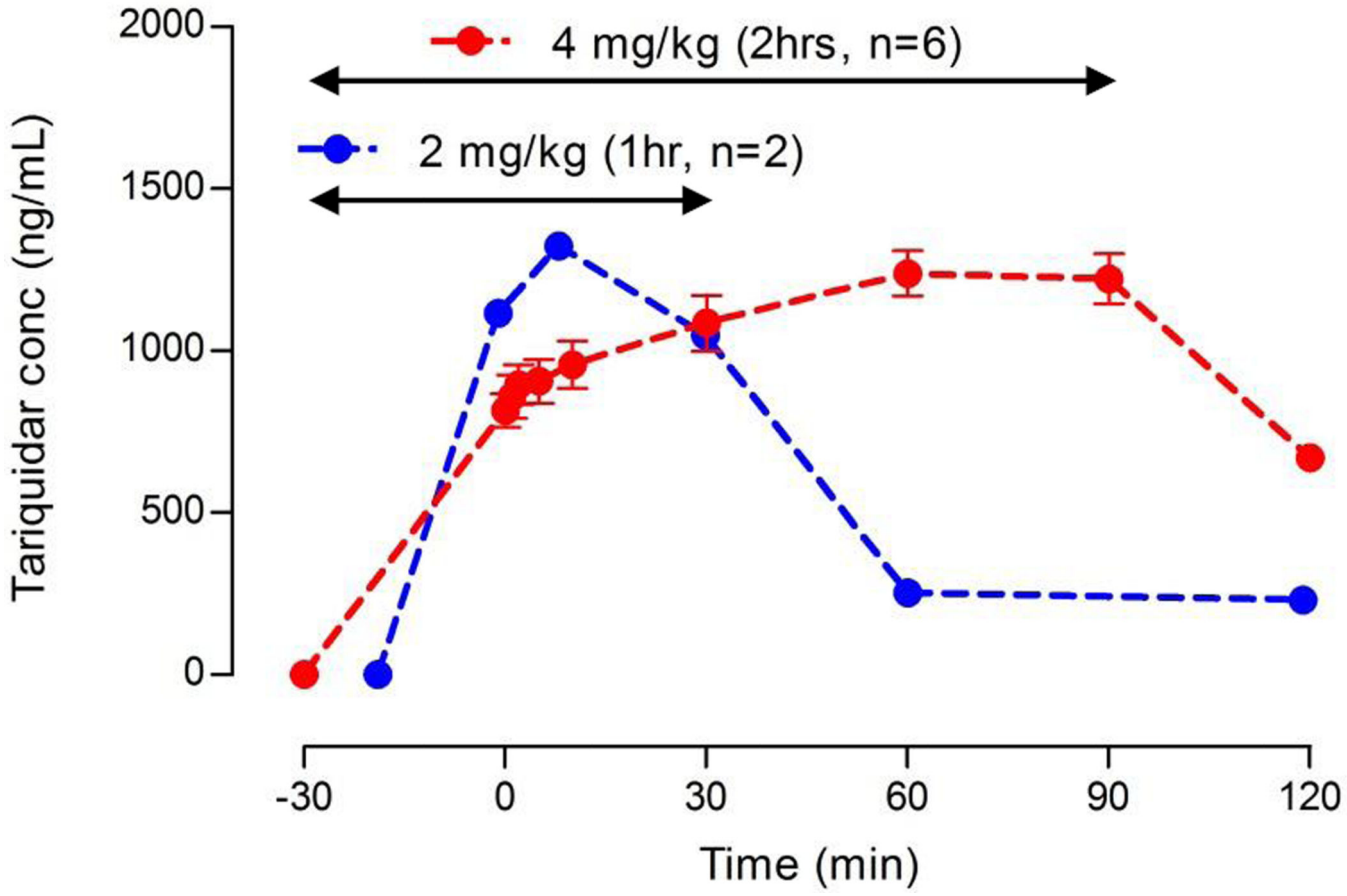

Figure 3.

Plasma tariquidar concentration measured by liquid chromatography-mass spectrometry (LC-MS/MS)(Kreisl et al., 2010). Infusion of *i.v.* tariquidar began 30 minutes prior to ^{18}F-FCWAY injection and continued for one hour (●, n=2) or two hours (○, n=6). Arrows indicate when the infusion stopped for the two conditions, respectively. Error bars represent standard error of the mean (SEM).

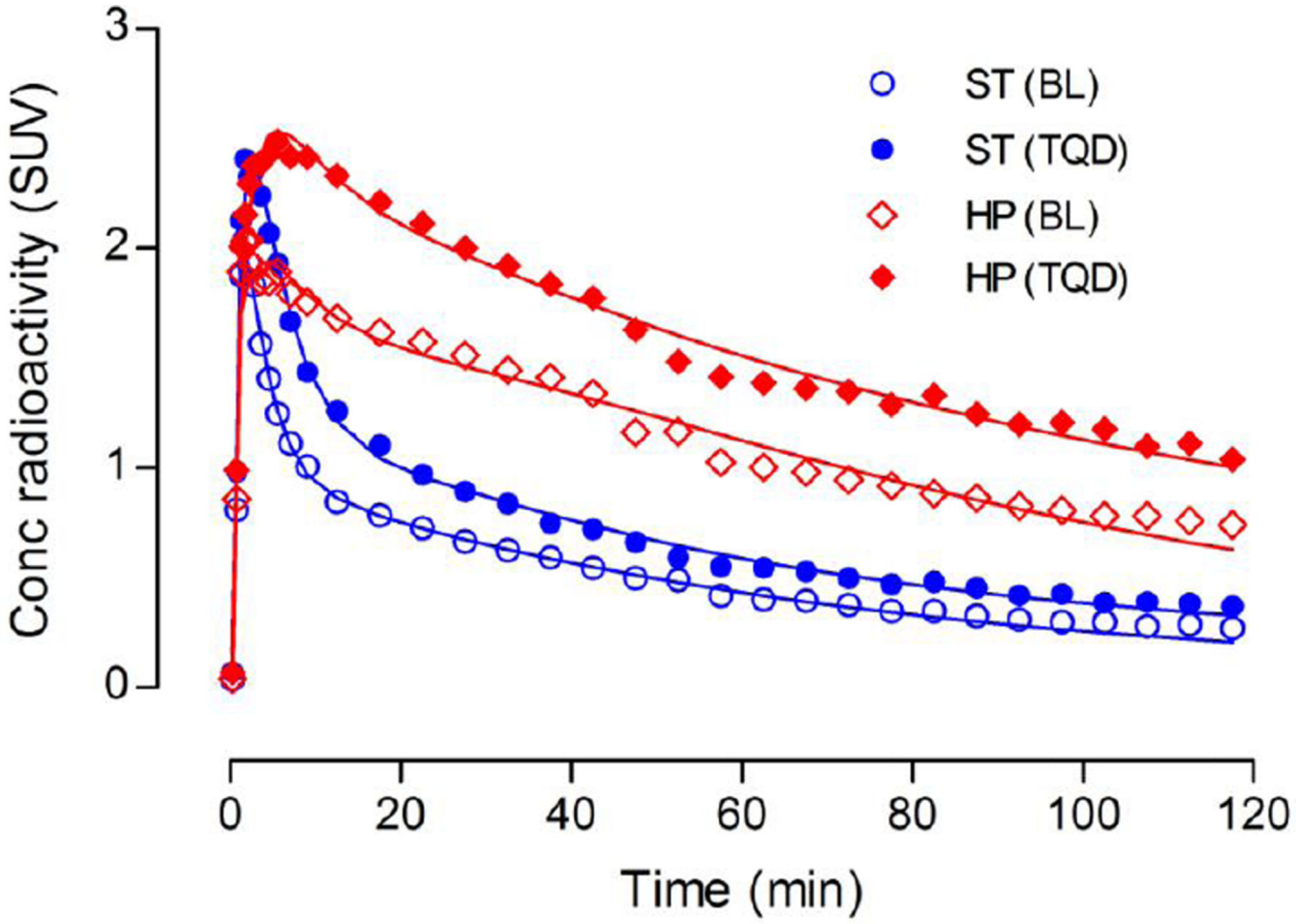

Figure 4.

Representative time activity curves of hippocampus (high 5-HT_{1A}) before (◇) and after (◆), and striatum (low 5-HT_{1A}) before (○) and after (●) *i.v.* tariquidar treatment. Solid lines are fitted curves with a three-compartment model (two-tissue compartments for ^{18}F-FCWAY and one metabolite compartment to account for ^{18}F-FC in the brain).

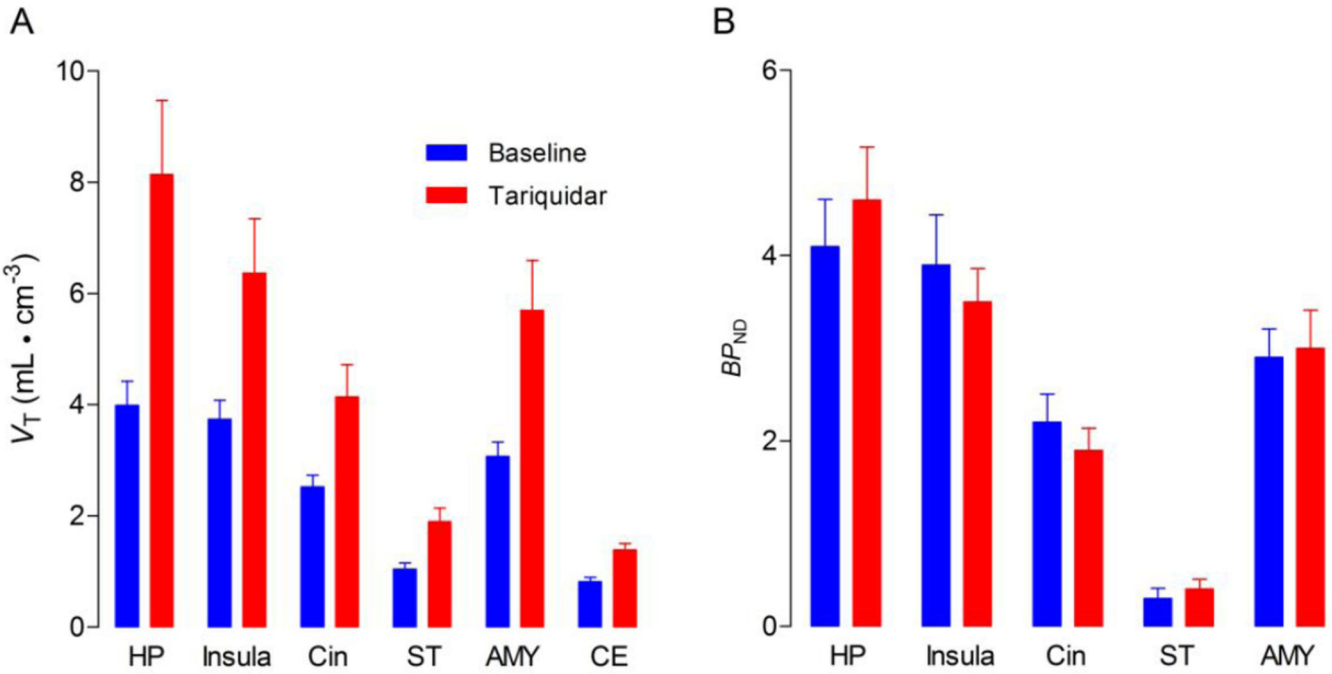

Figure 5.

(A) Mean distribution volume (V_T) increase after *i.v.* tariquidar treatment (n=8); (B) non-displaceable binding potential (BP_{ND}) changed insignificantly after *i.v.* tariquidar treatment. Error bar represents standard error of the mean (SEM). All regions were statistically significant (paired *t*-test, one-tailed, $p < 0.02$).

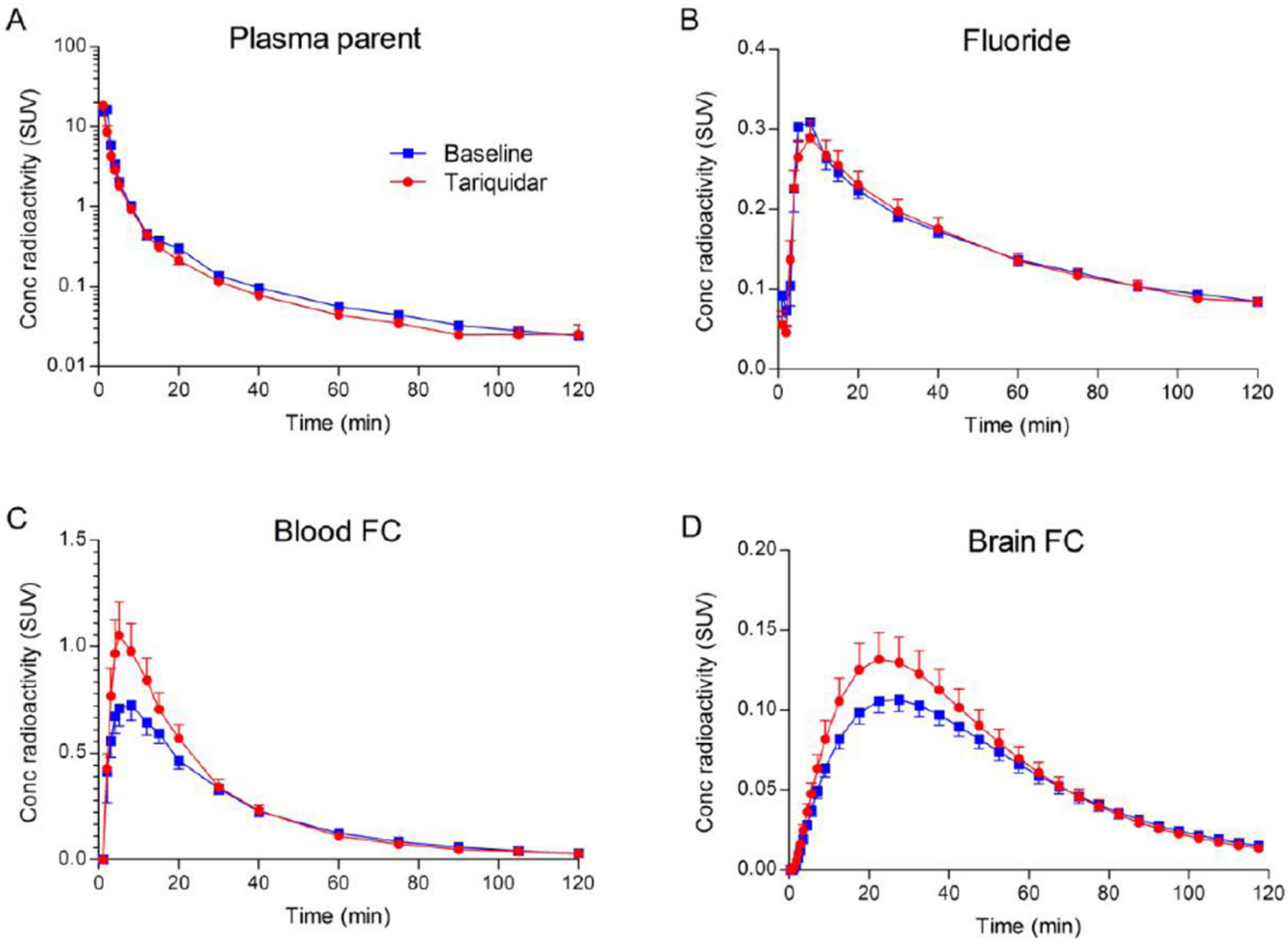

Figure 6.

Mean time activity curves across all subjects (n=8) for (A) plasma parent concentration; (B) plasma fluoride concentration; (C) plasma ^{18}F-FC concentration; and (D) estimated brain ^{18}F-FC concentration. Error bars represent standard error of the mean (SEM)..

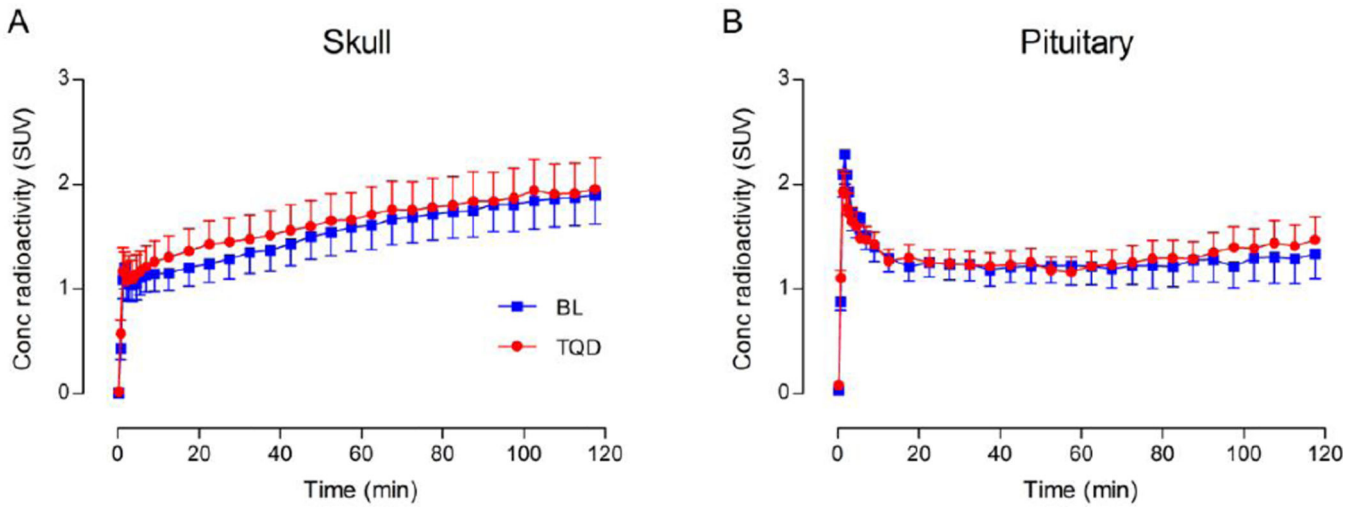

Figure 7.

No change in skull (A) or pituitary gland (B) uptake after *i.v.* tariquidar treatment (n=8). Both structures are outside the blood brain barrier.

Table 1

Percent inhibition of the fluorescent substrates by FCWAY under two concentrations for each of the three ABC efflux transporters. For each transporter, percent inhibition by an avid substrate was also shown for reference. A 0% inhibition indicates not a substrate and the larger the inhibition, the more avid a substrate is to the specific transporter.

	Percent Inhibition (%)	
	20 uM	**50 uM**
P-gp		
FCWAY	0.8	4.0
dLop	10.0	25.0
Mrp1		
FCWAY	3.1	5.4
MPPF	13.1	19.9
BCRP		
FCWAY	6.8	8.6
LM11	117.9	151.3