The AAPS Journal, Vol. 14, No. 1, March 2012 (© 2011)
DOI: 10.1208/s12248-011-9318-1

Research Article

Alteration in P-glycoprotein Functionality Affects Intrabrain Distribution of Quinidine More Than Brain Entry—A Study in Rats Subjected to Status Epilepticus by Kainate

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Received 15 September 2011; accepted 15 December 2011; published online 4 January 2012

Abstract. This study aimed to investigate the use of quinidine microdialysis to study potential changes in brain P-glycoprotein functionality after induction of status epilepticus (SE) by kainate. Rats were infused with 10 or 20 mg/kg quinidine over 30 min or 4 h. Plasma, brain extracellular fluid (brain ECF), and end-of-experiment total brain concentrations of quinidine were determined during 7 h after the start of the infusion. Effect of pretreatment with tariquidar (15 mg/kg, administered 30 min before the start of the quinidine infusion) on the brain distribution of quinidine was assessed. This approach was repeated in kainate-treated rats. Quinidine kinetics were analyzed with population modeling (NONMEM). The quinidine microdialysis assay clearly revealed differences in brain distribution upon changes in P-glycoprotein functionality by pre-administration of tariquidar, which resulted in a 7.2-fold increase in brain ECF and a 40-fold increase in total brain quinidine concentration. After kainate treatment alone, however, no difference in quinidine transport across the blood–brain barrier was found, but kainate-treated rats tended to have a lower total brain concentration but a higher brain ECF concentration of quinidine than saline-treated rats. This study did not provide evidence for the hypothesis that P-glycoprotein function at the blood–brain barrier is altered at 1 week after SE induction, but rather suggests that P-glycoprotein function might be altered at the brain parenchymal level.

KEY WORDS: blood–brain barrier; epilepsy; microdialysis; P-glycoprotein (P-gp); quinidine.

INTRODUCTION

P-glycoprotein (P-gp) is the best studied efflux transporter at the endothelial capillaries constituting the blood–brain barrier (BBB) (1,2). It is responsible for reduced brain distribution of many clinically used drugs. For drugs aimed at systemic targets this can be a benefit as the action of P-gp will help to reduce central side effects. P-gp might, however, also hamper the ability of drugs to reach target sites within the brain. For example, about 30–40% of all people with epilepsy do not become fully seizure free with current medications, even when treated at maximal tolerated doses. Lack of response in these individuals is not limited to a specific drug or drug class, but occurs with the complete range of anti-epileptic drugs (3,4). Thus, it has been hypothesized that increased P-gp expression in a subpopulation of people with severe epilepsy is, at least in part, responsible for pharmacoresistance by impairing drug access to the brain

There are many methods used to study drug distribution into the brain (5). However, most of these methods focus on total brain concentrations. Since brain tissue is rather lipophilic, total brain concentrations will often mainly reflect the lipophilicity of the drug, i.e., a lipophilic drug will have a higher total brain concentration than a more hydrophilic drug. If the transport across the BBB is of interest, methods that measure free concentrations in brain are needed, as a reflection of the fraction of the molecules in plasma that can pass the tightly connected cell membrane of the BBB. Microdialysis is the state-of-the-art methodology for measuring free drug concentrations in the brain. Transport processes at the BBB and the brain parenchyma can be separated by using microdialysis in combination with measurement of plasma and total brain concentrations (6,7).

Quinidine, a substrate with high affinity for P-gp, was used in the present study as a model substrate for determining P-gp functionality at the BBB. To investigate the specific contribution of P-gp at the BBB, rats were studied without or with co-administration of the P-gp inhibitor tariquidar (TQD). Quinidine concentrations were determined in arterial plasma and extracellular brain fluid for 7 h after the start of quinidine administration. In addition, quinidine concentration in brain tissue was determined at the end of the experiment.

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ABBREVIATIONS: BBB, Blood–brain barrier; ECF, Extra cellular fluid; ip, Intraperitoneal; OFV, Objective function value; P-gp, P-glycoprotein; PET, Positron emission tomography; SE, Status epilepticus; TQD, Tariquidar.

In rats, treatment with intraperitoneal (ip) injections of kainate leads to status epilepticus (SE) (8). After a latent period of 3–4 weeks post-SE, rats start to show spontaneous seizures. The kainate model in rats displays several features of temporal lobe epilepsy, the most common form of epilepsy frequently associated with pharmacoresistance, including an increase in P-gp expression (9,10). In a recent positron emission tomography (PET) study it was shown that the brain distribution volume of the PET P-gp substrate tracer (*R*)-[^{11}C] verapamil was increased in kainate-treated rats compared to control rats (11). However, unlike microdialysis, PET measures total concentrations and hence pharmacokinetics at the level of the BBB *versus* intrabrain cannot be separated.

The aim of the present study was first develop a microdialysis method for studies of drug transport across the BBB and intrabrain distribution, and second, to investigate the influence of kainate treatment and TQD pre-administration on quinidine pharmacokinetics with focus on P-gp functionality, both at the BBB and brain parenchyma.

MATERIALS AND METHODS

Chemicals and Solutions

Tariquidar (XR9576, TQD) was obtained from Xenova Group PLC (Cambridge, England) or API Services Inc. (Westford, USA), kainic acid, quinidine, quinidine sulfate dehydrate, quinidine hemi sulfate and quinine hemi sulfate from Sigma Aldrich (Zwijndrecht, The Netherlands), triethyl amine from Baker (Deventer, The Netherlands), boric acid and orthophosphoric acid 85% was from Merck (Darmstadt, Germany), methyl *tert*-butyl etherfrom Bioslove (Valkenswaard, The Netherlands), isoflurane from Pharmachemie BV (Haarlem, The Netherlands), 5% glucose in saline and saline from hospital pharmacy of the University Medical Centre Leiden (UMCL, Leiden, The Netherlands).

Perfusion fluid for microdialysis was a modified artificial cerebrospinal fluid solution previously described by Moghaddam *et al*. (12). In short, it was a water based solution containing 140 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, and 1.2 mM $CaCl_2$. For injections, kainate was dissolved in saline to a total concentration of 5 mg/mL; quinidine was dissolved in saline to a total concentration of 1.25 or 2.5 μg/μL and TQD was dissolved in 5% glucose in saline to a total concentration of 3.75 mg/mL.

For the HPLC analysis, quinidine was dissolved in methanol and diluted with water to a concentration of 100 μg/mL. This stock solution was further diluted in water (plasma and brain analysis) or perfusion fluid (microdialysate analysis) to construct calibration curves for quinidine analysis.

Animals

Adult male Sprague–Dawley rats (n=74, Harlan, Horst, The Netherlands) weighing 200–249 g on arrival were housed in groups of five to six per cage until treatment or surgery and thereafter housed individually. They were kept at a constant temperature of 21±1°C and at a 12 h light/dark cycle, in which white lights were switched on at 8:00 AM. Animals had unrestricted access to food (RM3 (E) DU, Special Diets Services B.V., Witham, Essex, England) and acidified water. Animal procedures were performed in accordance with Dutch laws on animal experimentation. All experiments were approved by the Ethics Committee for Animal Experiments of Leiden University (approval numbers UDEC08134 and UDEC08200).

Two groups of animals were studied: (1) untreated control rats and (2) kainate-treated rats. The number of animals in each group is shown in Table I. Of the initial 74 animals, 10 animals had to be excluded either because of technical reasons such as clogging of cannulae or irregularities in flow through the microdialysis probe, or due to death or insufficient recovery after kainate treatments.

The control group (n=48) underwent surgery approximately after 1 week of habituation and 7 days prior to microdialysis experiments. In short, rats were anesthetized via a nose mask with isoflurane, 4–5% induction and 1.5–2% maintenance, in oxygen 1 L/min. Blood cannulae (Portex Fine bore PE tubing, Smith Medical B.V., Rosmalen, The Netherlands) were implanted in the femoral artery for blood sampling and the femoral vein for drug administration. This was followed by insertion of a microdialysis guide with a dummy probe (CMA, Solna, Sweden), which was implanted in the hippocampus (AP-5.6, L4.6, V-2.5). Six of the control animals were used to determine the *in vivo* recovery of quinidine by retro dialysis (13). These animals had a microdialysis guide and dummy implanted 7 days prior to the experiment, but no blood cannulae.

The rats in group 2, were treated with saline (n=3), or kainic acid (n=16) to induce SE after approximately 1 week of habituation and 7 days prior to microdialysis experiments. Kainic acid was administered repetitively until full-blown seizures were seen (8). First, an initial dose of 10 mg/kg (2 mL/kg) i.p. was administered followed by 5 mg/kg (1 mL/kg) i.p. every 30–60 min until stage IV seizures according to Racine's

Table I. Number of Animals in the Different Treatment Groups

	Control rats + vehicle co-administration	Control rats + tariquidar co-administration	Kainate-treated + vehicle co-administration	Kainate-treated + tariquidar co-administration
Quinidine 12.5 μg/min over 4 h	7	6	–	–
Quinidine 25.0 μg/min over 4 h	8	8	–	–
Quinidine 100 μg/min over 30 min	9[a]	7[b]	8	8
In vivo retrodialysis	3	3	–	–

[a] Two of the nine animals were included from the second groups of rats (see text for further explanation)
[b] One of the seven animals were included from the second groups of rats (see text for further explanation)

scale (14) occurred, or when a total amount of 30 mg/kg kainic acid was reached. Six days later and 1 day prior to the microdialysis experiment, the animals underwent cannulation surgery and the implantation of the microdialysis probe as described in the section above.

After the microdialysis study, animals were euthanized by decapitation and the brain was removed. All samples, i.e., plasma, microdialysate and brain tissue were stored at −80°C until analysis

Pharmacokinetic Study in Rats

The dummy probe was replaced with the microdialysis probe 1 day (18–22 h) prior to the microdialysis experiment. On the day of the microdialysis study, the probe inlet was connected to the perfusion fluid syringe and the outlet to an automatic cooled fraction collector (Univentor microsampler 820, Univentor Ltd, Zejtun, Malta). When the rat was properly connected the perfusion rate was set to 2 μL/min and was kept on this rate throughout the experiment. Samples were collected for 15 min, i.e., the fraction collector turned every 15 min. The perfusion rate was checked by weighing each sample, and samples obtained at perfusion rates deviating from the set rate with more than 10% were excluded. The probes were perfused for at least 60 min (blank period) prior to the start of the quinidine intravenous infusion. Quinidine was infused at 10 μL/min (2.5 or 1.25 μg/μL) over 4 h or 40 μL/min (2.5 μg/μL) over 30 min. The number of animals in each dose group is shown in Table I. Half of the animals were treated with the P-gp inhibitor TQD, 15 mg/kg, 30 min before the start of the quinidine infusion. The other half was treated with 5% glucose in saline (vehicle) at the same time point.

In Vivo Retro Dialysis

Microdialysis probe recovery was determined in six rats through *in vivo* retro dialysis (15). The animals were each infused with one or two concentrations of quinidine in perfusion fluid. The quinidine concentrations were randomized and ranged between 20 and 500 ng/mL to cover the whole concentration range observed in the study. The probe recovery was calculated as described in Eq. 1.

$$\text{Extraction fraction} = \frac{C_{in} - C_{out}}{C_{in}} \quad (1)$$

Where C_{in} is the quinidine concentration in the perfusion fluid, C_{out} is the concentration in the dialysate and the "extraction fraction" loss of molecules to the tissue. It is assumed that the loss is equal to the gain, i.e., if 25% of molecules in the perfusion fluid are lost to the tissue it is assumed that a blank perfusion fluid, as used in the experiments, will pick up 25% of the molecules present in the tissue.

Quantification of Quinidine in Plasma, Dialysates, and Brain Tissue

Quinidine was measured using HPLC with fluorescence detection. A LC10-ADVP HPLC pump (Shimadzu, 's-Hertogenbosch, the Netherlands) using a flow of 1 mL/min. An Altima C-18 150×4.6 mm column (Grace Altech, Breda, the Netherlands) was used to elute quinidine. Detection was performed using a Jasco FP-1920 fluorescence detector (Jasco, de Meern, the Netherlands) set at 488 nm for excitation and 512 nm for emission. Injection was performed using a Waters 717 autosampler (Waters, Etten-Leur, the Netherlands). The injection volume was 20 μL. Data acquisition was performed using Empower Software (Waters, Etten-Leur, The Netherlands). The mobile phase consisted of acetonitrile and phosphate buffer (25 mM) with triethyl amine (10 mM) at a pH of 2.3 in a ratio of 17:83 (*v*/*v*) and 14/86 (*v*/*v*) for microdialysis and plasma samples, respectively.

Microdialysate was injected directly, without any pretreatment and measured dialysate concentrations were converted into brain extracellular fluid (brain ECF) concentrations by division with the extraction fraction obtained using Eq. 1.

$$C_{ECF} = \frac{C_{dial}}{\text{Extraction fraction}} \quad (2)$$

To 20 μL plasma, 50 μL of the internal standard quinine (Sigma Aldrich, Zwijndrecht, the Netherlands) was added in a concentration of 500 ng/mL.

After homogenization with 200 μL of borate buffer pH 10, 5 mL of methyl *tert*-butyl ether was added. After vortexing, centrifugation, and freezing of the aqueous layer, the organic phase was evaporated to dryness. The extracts were reconstituted in 100 μL of mobile phase and centrifuged at 4,000×*g* during 5 min. The clean plasma extracts were injected using a mobile phase with an acetonitrile/buffer ratio of 1:6. Calibration was performed using 20 μL aliquots of quinidine in concentrations of 5, 10, 20, 50, 100, 200, 500, 1,000, 2,000, and 5,000 ng/mL which were added to 20 μL blank plasma.

Quinidine concentration in brain tissue was analyzed by the following steps: whole brain was homogenized in phosphate buffer at pH 7.4. Per gram brain, 5 mL of buffer was added (50 mM). After addition of the internal standard quinine to 0.6 mL of the homogenate, 100 μL of sodium hydroxide 1 M was added and mixed thoroughly. Tertiary butyl methyl ether was added in a volume of 5 mL. After vortexing for 5 min and centrifugation, 4 mL of the supernatant was transferred to a clean glass tube and 100 μL of phosphoric acid 30 mM was added. After another 5 min of vortexing followed by centrifugation at 4,000×*g* for 10 min, the supernatants were aspirated and discarded. The remaining aqueous phase was centrifuged for 10 min at 11,000×*g*. An aliquot of 40–60 μL was transferred to clean glass vials and 20 μL was introduced into the HPLC system, using a mobile phase with an acetonitrile/buffer ratio of 1:6.

Precision and accuracy of the determination of quinidine in plasma and brain were below the 15% level. The precision and accuracy of quinidine measurement in microdialysate were less than 10%. Limit of quantification was 5 ng/mL for plasma, 20 ng/mL for brain, and 1 ng/mL for dialysate.

Data Analysis

Analysis of quinidine pharmacokinetics was performed using nonlinear mixed effects modeling in NONMEM VI (GloboMax LLC, Hanover, MD, USA). The phamacokinetic model was constructed in three steps. In the first step, a pharmacokinetic model for quinidine plasma concentrations

was developed. One, two, and three compartment models were evaluated. In the second step the model was extended to include brain ECF concentrations, while allowing the plasma estimates to change freely from the values obtained in the first step. Again, one and two compartment models were evaluated. The final step was to also include the total brain concentrations that were obtained at the end of the experiments.

Data from all rats were processed simultaneously. The subroutine ADVAN 6 and first-order conditional estimation with interaction were used throughout the modeling procedure. Model selection was based on the objective function value (OFV) with the lowest value corresponding to the best model. For nested models, OFV reductions of 3.83, 6.63 and 10.83 units correspond to improved fits at $p<0.05$, $p<0.01$, and $p<0.001$ levels, respectively. Further, model parameter uncertainty and visual analysis using software Xpose 4 (16) implemented in R 2.7.1 (The R foundation for Statistical Computing) accessed from Census (17) was used to assess the model performance. For example, predicted concentrations were plotted against measured concentrations and conditional weighted residuals (18) were calculated and plotted against time and concentration.

The inter-individual variation of a parameter was described by the exponential variance model:

$$\theta_i = \theta_{\text{pop}} \cdot \exp(\eta_i) \quad (3)$$

where θ_i is the parameter in the ith animal, θ_{pop} the parameter in a typical animal and η_i the inter-animal variability, which is assumed to be normally distributed around zero with a standard deviation ω. Equation 3 provides a means to distinguish the parameter value for the ith animal from the typical value predicted from the regression model. Inter-individual variation was investigated for all parameters, but incorporated only for those parameters for which it significantly ($p<0.01$, OFV reduction of 6.63 units) improved the model.

Pre-administration (TQD or vehicle), rat group (control or kainate-treated), infusion time, quinidine dose, recovery time since surgery and animal weight on the experimental day were defined as covariates to study their effects on the parameter estimates. A stepwise forward addition and backward deletion approach was applied to test the significance ($p<0.01$) for covariate inclusion. The residual variability was described with proportional error models for each compartment.

Performance of the population pharmacokinetic model was evaluated using 100 additional bootstrap replicates (re-sampling with replacement) of the data by fitting the final model to them.

RESULTS

Quinidine Plasma and Brain Profiles

Quinidine plasma and brain ECF concentration profiles for the groups of rats that were infused with 100 µg/min over 30 min are shown in Fig. 1. The brain uptake of quinidine without TQD pretreatment was in general low but was increased by pretreatment with TQD.

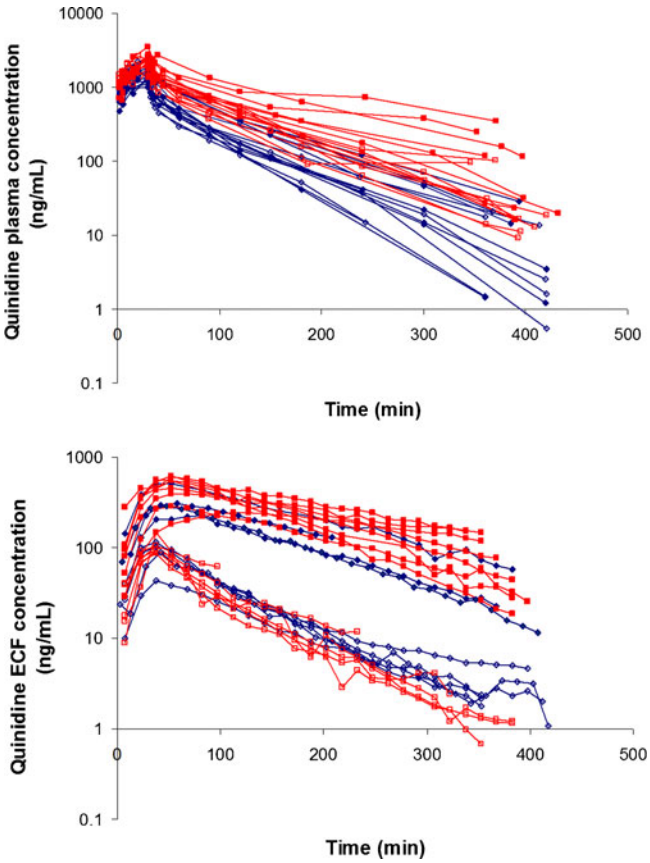

Fig. 1. Quinidine concentrations in plasma (*upper panel*) and brain ECF (*lower panel*) during and after an intravenous infusion of 100 µg/min over 30 min. *Blue* and *red symbols* are saline- and kainate-treated rats, respective. *Open* and *closed symbols* are without and with tariquidar treatment, respectively

Development of the Pharmacokinetic Model

Quinidine kinetics in both plasma and brain were analyzed using mixed effects modeling in NONMEM. The final model including covariate effects is shown in Fig. 2. Both the plasma and brain profiles were best described with two compartment models. As a first step, only plasma was modeled and as a second step the brain ECF and total brain compartments were included. When the ECF and total brain compartments were included, only minor changes were observed for the plasma parameter estimates (Table II). The model diagnostics plots, separated for plasma and brain ECF concentrations, are shown in Fig. 3.

It was not possible to estimate Q_{in}, Q_{out}, and V_{br} (Fig. 2) independently with good precision and thus the model was simplified to include a correlation between Q_{in} and Q_{out}:

$$Q_{\text{in}} = f_1 \cdot Q_{\text{out}} \quad (4)$$

where f_1 describes the correlation between Q_{out} and Q_{in}. In the final model, Q_{out} was fixed to a value of 10.8 mL/min as it was observed in the bootstrap analysis that the volume of distribution V_{br1} and Q_{out} were to some extent correlated, i.e. the ratio between the two parameters was stable, but for some of the data sets generated in the bootstrap analysis V_{br1} was estimated to an unrealistically high value. The value of

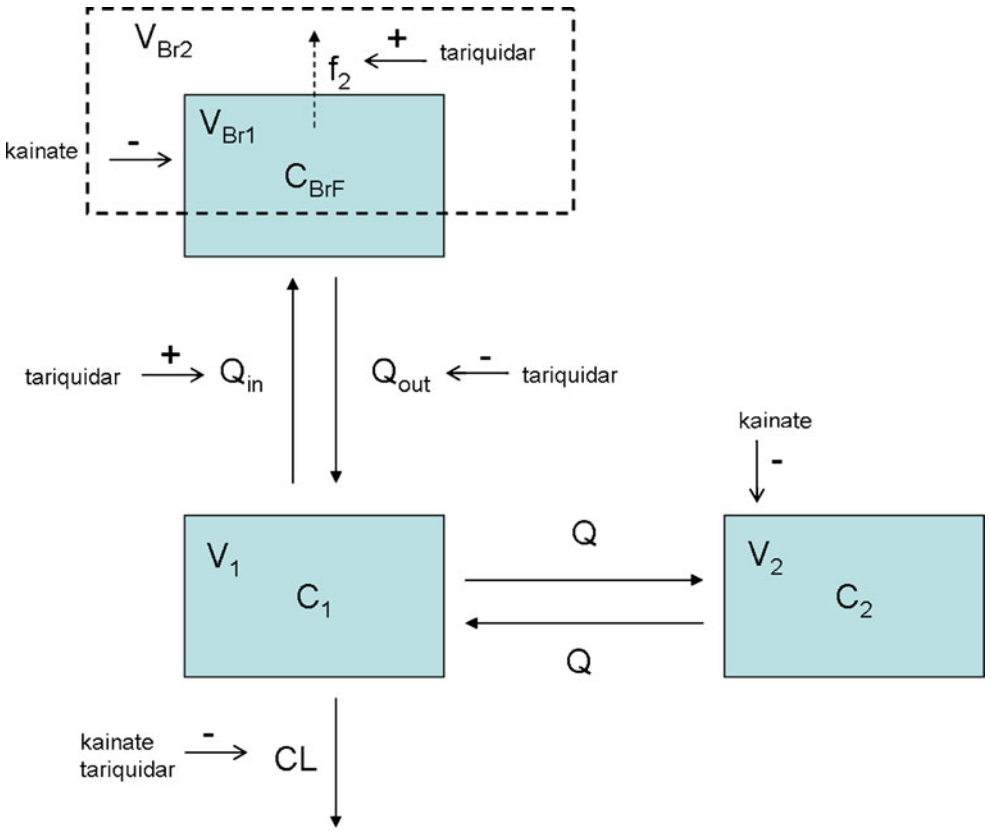

Fig. 2. Final pharmacokinetic model including covariates. C_1, C_2, C_{BrF}, and C_{BrT} are the quinidine concentrations (in nanograms per milliliter) in central plasma, peripheral tissue, unbound brain, and deeper brain compartment, respectively. V_1, V_2, and V_{Br} are the volumes of distribution (in milliliters) in central plasma, peripheral tissue and unbound brain, respectively. CL, Q, Q_{in}, and Q_{out} are the clearances (in milliliters per minute) from plasma, between the two plasma compartments, into the brain and out from the brain, respectively. Q_{in} was defined as a fraction of Q_{out}, $Q_{in} = f_1 \bullet Q_{out}$. The distribution from the free brain compartment to the deeper brain compartment was described with a fraction, f_2, so that $C_{BrT} = f_2 \bullet C_{BrF}$. The covariate analysis showed that kainate treatment decreased the systemic clearance, the volume of distribution in peripheral tissue and brain while tariquidar co-administration decreased the systemic clearance, increased the quinidine clearance into the brain, decreased the clearance out from the brain and increased the distribution from the unbound brain compartment to the deeper brain compartment

10.8 mL/min was obtained with the final model when Q_{out} was kept free during the estimation.

The concentration in the total brain compartment (C_{br2}) was assumed to be correlated with the concentration in the first brain compartment (C_{br1}) as:

$$C_{br2} = f_2 \cdot C_{br1} \tag{5}$$

In the present study, measurements were obtained in both of these brain compartments. The concentration in the first brain compartment (C_{br1}) was the free quinidine concentration in brain ECF, while the concentration in the second brain compartment (C_{br2}) was the concentration of quinidine in total brain tissue, i.e., intra- and extracellular fluid as well as brain tissue.

Inter-individual variation was found for structural model parameters CL, V_1, V_2, V_{Br}, and Q_{in} (Table II). The covariate analysis showed no correlation between parameter estimates and quinidine dose, infusion time, recovery time after surgery or recovery time after probe insertion. When only plasma was fitted the OFV was reduced by 9.5 units ($p<0.01$) when animal weight was added as a covariate for clearance. However this covariate was not significant when the model was extended also to include brain compartments (Table III).

Analysis of Influence of TQD and Kainate Treatment

Both TQD pre-administration and kainate treatment were treated as categorical covariates, i.e., they were assigned a value of 0 or 1. The final parameter equations, including covariates were defined as:

$$\theta_i = \theta_{pop} \cdot \exp(\eta_j) \cdot \theta_{covar}^{\text{COVARIATE (1 or 0)}} \tag{6}$$

Where θ_{covar} describes the influence of TQD pre-administration or kainate treatment on parameter estimate θ_{pop} (Eq. 6). In Eq. 6, the exponent COVARIATE was assigned a value of 1 for TQD pre-administration and kainate treatment and a value of 0 for vehicle co-administration and control rat. The θ_{covar} estimate therefore represents the fractional change between TQD and vehicle pre-administered or kainate-treated and untreated groups. Equations for all model parameters are given in Table IV.

Table II. Quinidine Pharmacokinetic Model Parameter Estimates

Objective function	Estimation			Bootstrap		Estimation plasma only	
	−2898			−2964		−695	
Parameter	Value	CV%	Shrinkage%	Value	CV%	Value	CV%
Fixed effects							
CL (mL/min)	32.1	5.1		32.3	5.2	29.9	4.8
V_1 (mL)	146	42		145	32	188	27
Q_2 (mL/min)	66.6	11		66.8	11	73.3	10
V_2 (mL)	1840	6.5		1851	6.1	1630	4.4
Q_{out} (mL/min)	10.8	N.A.		10.8	N.A.		
f_1	0.0619	8.2		0.0621	8.3		
V_{Br} (mL)	269	14		281	15		
f_2	8.67	2.7		9.51	30		
Covariates							
Kainate (CL)	0.537	8.5		0.539	8.4	0.556	8.8
Kainate (V_2)	0.678	7.0		0.676	5.9	0.746	6.4
Kainate (V_{Br})	0.592	13		0.591	12		
Tariquidar (CL)	0.835	6.8		0.835	6.5		
Tariquidar (Q_{out})	0.366	14		0.376	16		
Tariquidar (Q_{in})	2.65	17		2.73	18		
Tariquidar (f_2)	5.66	33		5.65	29		
Weight (CL)	N.A	N.A		N.A	N.A	0.967	46
Inter-individual variability							
η(CL)	0.0682	20	6	0.0612	19	0.0731	18
$\eta(V_1)$	1.92	30	18	2.03	34	1.61	29
$\eta(V_2)$	0.0686	34	15	0.680	32	0.0231	50
$\eta(Q_{in})$	0.101	19	13	0.0965	19		
$\eta(V_{br})$	0.196	31	16	0.183	44		
Propotional residual error							
σ^2(plasma)	0.270	7.0		0.271	7.6	0.233	5.6
σ^2(brain ECF)	0.185	9.4		0.183	9.1		
σ^2(total brain)	0.694	21		0.636	22		

TQD Treatment

TQD pre-administration increased Q_{in} 2.65-fold, decreased Q_{out} 2.73-fold (1/0.366) and increased f_2, i.e., the correlation between C_{br1} and C_{br2}, 5.66-fold (see Table II). Since it was assumed that there was a correlation between Q_{in} and Q_{out}, the model was also fitted with only TQD influence on either Q_{in} or Q_{out}, but in both cases the OFV increased by >25 units(see Table III). Taken together this indicates that TQD pre-administration can increase the free brain concentrations 7.2-fold (2.65×1/0.366=7.2) by acting on both Q_{in} and Q_{out}. The increase in *total* brain concentrations was much larger, about 40-fold (2.65×1/0.366×5.66=41) indicating that TQD also increased the distribution from the brain ECF to brain tissue.

Kainate Treatment

Typically, three injections, i.e., a cumulative dose of 20 mg/kg, were needed to reach stage IV or V seizures according to Racine's scale (14) and SE was usually reached within 20 min after the last injection. Without exception all rats reached this stage. Rats usually displayed seizures for 6–12 h, with the most intense and frequent seizures occurring during the first 2 h. The seizures were not interrupted by any anti-epileptic drug treatment. The mortality was less than 10%. Due to the design of this study it was not possible to determine whether these animals eventually would have developed spontaneous epilepsy, but the signs and symptoms during and after SE were very similar to those observed in previous studies where animals did develop epilepsy. Further, some of the kainate-treated animals had seizures during the microdialysis experiments. The seizures were usually provoked by stress, i.e., the rats experienced seizures during or just after being connected to the perfusion fluid syringe and sample collector.

Kainate treatment was found to be a significant covariate for the systemic clearance (CL) and volume of distribution in the peripheral plasma (V_2; see Fig. 2 and Table II). Kainate treatment also reduced the V_{br} 1.69-fold (1/0.59; Table II).

Further, the total brain-to-plasma concentrations ratios at the end of experiments were lower in kainate-treated than in control animals. This brain-to-plasma ratio was (average ± standard deviation) 0.9±0.6 in control animals and 0.6±0.4 in kainate-treated animals pre-administered with only vehicle. In TQD pre-administrated animals the ratios were 114±80 in control animals and 45±29 in kainate-treated animals. However, in the population model kainate as a covariate for f_2, i.e., the distribution into the deeper brain compartment, did not result in a significant drop of OFV (Table III).

In Vivo Retro Dialysis

A typical profile of retrodialysis in one animal is shown in Fig. 4. The probe concentration recovery was not

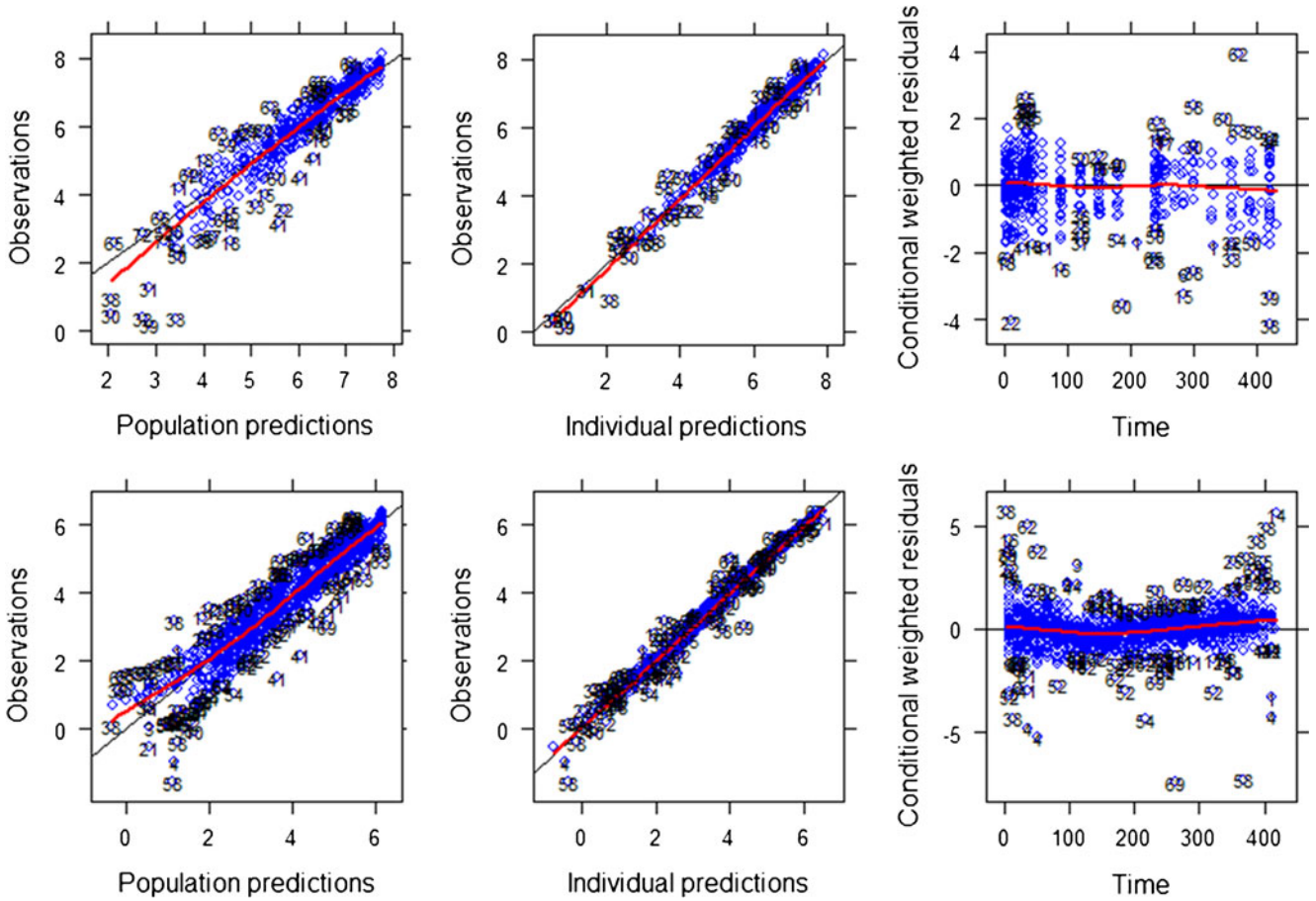

Fig. 3. Model diagnostics for plasma (*upper panel*) and brain extracellular fluid (*lower panel*). The model described the data well, as the residuals in the *left* and *middle columns* are distributed around the line of identity (*thin black line*), with *dots* closer to this line in the middle column as the inter-individual variability is taken into account in these two plots. *Plots in the right column* show the conditional weighted residuals versus time and these are also randomly scattered around zero, thus showing no trend over time

Table III. Model Development Table

Model	ΔOFV (compared to final model)	Comment
1-comp plasma	1,133	Increase >6.63 in OFV → significant worse fit than final model
Without covariates		
Kainate (CL)	40.38	Increase >6.63 in OFV → significant worse fit than final model
Kainate (V_2)	14.36	Increase >6.63 in OFV → significant worse fit than final model
Kainate (V_{Br})	9.91	Increase >6.63 in OFV → significant worse fit than final model
Tariquidar (CL)	49.25	Increase >6.63 in OFV → significant worse fit than final model
Tariquidar (Q_{out})	28.93	Increase >6.63 in OFV → significant worse fit than final model
Tariquidar (Q_{in})	25.41	Increase >6.63 in OFV → significant worse fit than final model
Tariquidar (f_2)	19.22	Increase >6.63 in OFV → significant worse fit than final model
With covariates		
Weight (CL)	−0.289	Non-significant change from final model
Weight (V_1)	−0.123	Non-significant change from final model
Weight (V_2)	−0.09	Non-significant change from final model
Kainate (Q)	0.00	Non-significant change from final model
Kainate (V_1)	−5.09	Non-significant change from final model
Kainate (Q_{in})	−5.32	Non-significant change from final model
Kainate (Q_{out})	−6.42	Non-significant change from final model
Kainate (f_2)	−0.73	Non-significant change from final model
Tariquidar (V_1)	−5.19	Non-significant change from final model
Tariquidar (V_2)	−0.02	Non-significant change from final model
Tariquidar (Q)	−3.11	Non-significant change from final model
Tariquidar (V_{Br})	−2.485	Non-significant change from final model

Table IV. Model Parameter Equations

Plasma
$CL = \theta_1 \cdot \theta_2^{KAINATE} \cdot \theta_3^{TARIQUIDAR} \cdot \exp(\eta_1)$
$V_1 = \theta_4 \cdot \exp(\eta_2)$
$Q = \theta_5$
$V_2 = \theta_6 \cdot \theta_7^{KAINATE} \cdot \exp(\eta_3)$
Brain
$Q_{out} = \theta_8 \cdot \theta_9^{TARIQUIDAR}$
$V_{Br} = \theta_{10} \cdot \theta_{11}^{KAINATE} \cdot \exp(\eta_4)$
$f_1 = \theta_{12}$
$Q_{in} = f_1 \cdot \theta_8 \cdot \theta_{13}^{TARIQUIDAR} \cdot \exp(\eta_5)$
$f_2 = \theta_{14} \cdot \theta_{15}^{TARIQUIDAR}$

Where covariates KAINATE and TARIQUIDAR are 1 for treated animals and 0 for non-treated animals

dependent on quinidine concentrations (20–500 ng/mL) nor on treatment with TQD. As can be seen in Fig. 4, there was an instantaneous equilibrium (no lag-time) when the concentration in the perfusion fluid was changed. The loss from the perfusion fluid was average 24% (standard deviation 5.8%) and this value was used as the extraction fraction in Eq. 2 to convert quinidine microdialysate concentrations to brain ECF concentrations.

DISCUSSION

Quinidine concentrations in the brain were very low in both kainate- and saline-treated rats. Remarkably, kainate-treated rats tended to have a lower total brain concentration but a higher brain ECF concentration of quinidine than saline-treated rats. This suggests merely that the P-gp function in kainate-treated rats is altered at the parenchymal level rather than at the BBB. TQD pre-administration increased the brain ECF brain concentration 7.2-fold and the total brain concentration about 40-fold, supporting an important role for P-gp in intrabrain distribution. The effect of TQD was similar in control and kainate-treated rats.

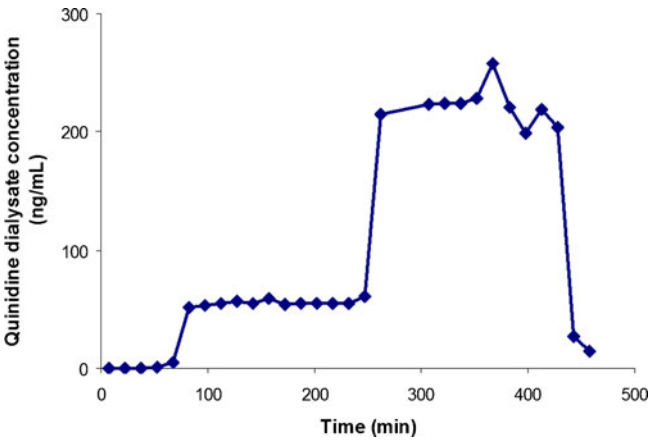

Fig. 4. An *in vivo* retrodialysis profile for a typical animal. Blank perfusion fluid was perfused during 0–60 min. Then, a perfusion fluid containing 71 ng/mL quinidine was perfused up to 240 min. From 240 to 420 min, the perfusion solution contained 273 ng/mL quinidine. After 420 min, the animal was again perfused with blank perfusion fluid. Samples we obtained every 15 min

Compared to many other methods, microdialysis has the important advantage of measuring the free (ECF) brain concentration (6,7). This concentration is relevant for estimating the transport across the BBB (19). In addition to the free concentrations that were measured with microdialysis over the whole study time, total quinidine concentrations were measured at the end of the experiment. Hence, the combination of microdialysis, plasma and total brain tissue concentrations produced a very rich data set including information on both transport across the BBB and distribution within the brain.

TQD treatment was used to inhibit the P-gp function. As mentioned, this resulted in a 7.2-fold increase in brain ECF-to-plasma concentration ratio and an approximately 40-fold increase in total brain-to-plasma concentration ratio. PET studies have shown 10–15-fold increase in brain-to-plasma concentrations of the P-gp substrate (*R*)-[^{11}C]verapamil after inhibition with 15 mg/kg TQD (11,20). PET data can be compared to total concentrations and thus, the present study indicates that quinidine might be a better model drug to study P-gp functionality than verapamil, as a large difference in concentration between baseline and P-gp inhibition states is desirable for a good P-gp function marker, regardless of whether PET or microdialysis is used. Another possible conclusion is that microdialysis as a method is more sensitive than PET to assess changes in P-gp functionality as it measures the free concentration, while the PET signal is not solely reflecting on processes at the BBB but also distribution within the brain. In the future, it would be very interesting to use ^{11}C-labeled quinidine as a PET tracer to be able to compare the read-out from microdialysis and PET. Initially, verapamil was also tried in the microdialysis setting, but due to extensive sticking of verapamil to the microdialysis tubing and probes this was not possible. When designing the present study, use of an anti-epileptic drug as a model drug was considered, but since a large difference between normal P-gp function and increased/decreased P-gp function was desired, it was concluded that a strong P-gp substrate, such as quinidine, was the best way to demonstrate proof of concept. In addition, quinidine as opposed to many other P-gp substrates, show no interaction with the other major efflux transporters, e.g., breast cancer resistant protein (21,22).

All brain tissue samples were obtained at approximately the same time point, i.e., at the end of the experiment 7 h after the start of quinidine infusion, at which equilibrium was assumed to be reached. However, rate constants for distribution to and from the total brain compartment could not be estimated based on the present data set. Therefore, concentration of quinidine in brain tissue was in the final pharmacokinetic model described as a multiple (f_2) of the free brain ECF concentration. Previous studies (unpublished data) in our lab have showed that equilibrium between brain ECF and total brain concentrations is very rapid and hence even if brain samples had been obtained at different time points it would still have been possible to use the present model structure. The result showed that quinidine concentration is 8.7-fold higher in brain tissue than in brain ECF.

In epilepsy, it has been hypothesized that P-gp expression at the BBB is upregulated and hence contributes to the development of pharmacoresistance (23–28). Therefore, it

was also of interest to investigate the P-gp function in kainate-treated rats, a frequently used model for temporal lobe epilepsy, and compare quinidine pharmacokinetics in this epilepsy model both at baseline and after P-gp inhibition to naïve control rats. Rats subjected to kainate induced SE become truly epileptic only after 3–4 weeks when spontaneous seizures start to occur (8), but the most prominent increase in P-gp expression has been reported between 1 and 7 days after SE (9,10,29–31). Thus, although seizures were not yet present at 1 week after induction of SE by kainate (apart from a few incidental observations), it can be assumed that at this time point after SE the brain both exhibits increased P-gp expression and is progressing towards the epileptic state. In a previously published study using the same epilepsy model, it was shown that the brain glucose metabolism, measured with ^{18}F-fluorodeoxyglucose, was significantly lower in kainate-treated rats 7 days post-SE than in controls (11). It is a common clinical observation that ^{18}F-fluorodeoxyglucose uptake is decreased in epileptic brain tissue (32). This suggests that the kainate model in rats mimics some functional changes that have been observed in the human epileptic brain.

Kainate treatment influenced the plasma pharmacokinetics of quinidine by decreasing the clearance (CL) and the peripheral volume of distribution (V_2). There was no effect on the Q_{in} and Q_{out}, but kainate treatment decreased the volume of distribution in the brain, V_{br}. A decrease in V_{br} suggests higher concentrations of quinidine in the brain, which may appear to be a contradiction to the notion that kainate treatment should result in upregulation of P-gp and thus rather a decrease in brain concentrations. However, P-gp is not only expressed at the endothelial cells of the BBB but also at other cell types such as astrocytes and pericytes (33–35). There are studies showing that breakdown of BBB function may be a consequence of an inflammatory response and that inflammation is a cause for P-gp upregulation (33). In the normal healthy brain, P-gp is probably not an important player in the brain beyond the endothelial cells, but after SE, an inflammatory reaction could lead to upregulation of P-gp not only in endothelial cells, but also in glial cells and neurons and these may thus act as a "second line of defense in addition to the BBB" (34–36). Total brain concentrations appeared to be lower in kainate-treated although this effect did not reach significance in this study, probably due to a large variability in the different rat groups. In this context, the findings of Bankstahl *et al.* are of interest, as they reported that, after SE, total brain-to-plasma ratio of phenytoin in rats was lower, while the opposite was found in the brain dialysate to plasma ratio (30). Phenytoin is considered to be a (weak) P-gp substrate (37) and hence the results by Bankstahl *et al.* are in line with the results from the present study, i.e., that SE decreases the total brain concentrations and increases the free brain concentrations.

CONCLUSION

Quinidine uptake in the brain was very low in both kainate- and saline-treated rats. The effect of TQD was similar in control and kainate-treated rats and TQD treatment increased total quinidine brain concentrations more than ECF concentrations. Kainate-treated rats tended to have a lower total brain concentration and a higher brain ECF concentration of quinidine than saline-treated rats. Thus, this study did not provide evidence for the hypothesis that P-gp function is altered at the endothelial capillaries constituting the BBB at 1 week after SE induction. However, the study indicated that P-gp functionality might be altered at the brain parenchyma.

ACKNOWLEDGMENTS

We thank Robin Hartman, Maaike Labots, and Nathalie Doorenweerd for help with the animal surgery and microdialysis experiments. This work was supported by the EU 7th framework program EURIPIDES (FP7/2007-2013 under grant agreement no. 201380).

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