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Short Communication

Use of Subcutaneous and Intraperitoneal Administration Methods to Facilitate Cassette Dosing in Microdialysis Studies in Rats[S]

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ABSTRACT

Microdialysis is a powerful technique allowing for real-time measurement of unbound drug concentrations in brain interstitial fluid in conscious animals. Use of microdialysis in drug discovery is limited by high resource requirement and low throughput, but this may be improved by cassette dosing. Administering multiple compounds intravenously of diverse physiochemical properties, it is often very challenging and time consuming to identify a vehicle that can dissolve all of the compounds. To overcome this limitation, the present study explores the possibility of administering a cassette dose of nine diverse compounds (carbamazepine, citalopram, desmethylclozapine, diphenhydramine, gabapentin, metoclopramide, naltrexone, quinidine, and risperidone) in suspension, rather than in solution, by intraperitoneal and subcutaneous routes, and determining if this is a viable option for assessing blood-brain barrier penetration in microdialysis studies. Repeated hourly subcutaneous dosing during the 6-hour microdialysis study allowed for the best attainment of distributional equilibrium between brain and plasma, resulting in less than a 2-fold difference in the unbound brain to unbound plasma concentration ratio for the cassette dosing method versus discrete dosing. Both subcutaneous and intraperitoneal repeated dosing can provide a more practical substitute for intravenous dosing in determining brain penetration of a cassette of diverse compounds in brain microdialysis studies. The results from the present study demonstrate that dosing compounds in suspension represents a practical approach to eliminating the technical challenge and labor-intensive step of preparation of solutions of a mixture of compounds and will enable the use of the cassette brain microdialysis method in a central nervous system drug discovery setting.

Introduction

An important property of drugs is the rate and extent of blood-brain barrier (BBB) penetration. This is determined by physiochemical properties such as size, charge, polarity, and lipophilicity, as well as the drug's affinity for influx and efflux transporters (Levin, 1980; Chikhale et al., 1994; Lee et al., 2001; Abraham, 2004; Liu et al., 2008). The property of brain penetration is critical for central nervous system targets, in which efficacy can only be achieved if the compound reaches brain tissue and engages with the target. There is also a more general implication for all drugs: on- or off-target central nervous system activity in relation to free drug in brain tissue, according to the free drug hypothesis (Brodie et al., 1960; Tillement et al., 1988; Liu et al., 2014). Therefore, it is advisable to have an understanding of brain penetration at an early stage of structure-activity relationship.

There are multiple methods currently employed for screening BBB penetration: some indirect and some direct. In silico methods use physiochemical properties, measured or predicted, to estimate permeability. In vitro screening methods such as permeability measurement in cell monolayers or cells plated on transwell devices are commonly employed (Nicolazzo et al., 2006). Screening usually stops short of in vivo methods for economical as well as ethical reasons. Furthermore, assessing BBB permeability in preclinical species requires destructive sampling. While some studies have used large numbers of animals to characterize kinetics of brain penetration (Chow et al., 2011), in an industry setting, a single time point is often used with the assumption that steady-state conditions are reached.

One means of averting this assumption is the use of brain microdialysis to indirectly measure brain concentrations in real time in live, conscious animals (de Lange et al., 1994; Liu et al., 2009). Unfortunately, this method requires surgical procedures that render it costly in the event that compounds are assessed discretely. Some efforts have been made to increase throughput of microdialysis studies by using cassette dosing (Deshmukh et al., 2015), but these efforts have currently been confined to intravenous bolus and infusion dosing. In many cases in drug discovery research, compounds with very diverse physiochemical properties are being tested, which makes finding a suitable vehicle to dissolve the compounds very difficult. This ultimately results in the use of doses that are very low, and for compounds with low probe recovery or low BBB penetration, dialysate concentrations are likely to fall below the limit of quantitation, which does not allow for a quantitative determination of brain penetration.

Identifying a suitable, nontoxic vehicle that can dissolve all of the compounds at a sufficient concentration for a brain microdialysis study represents a main challenge in the application of cassette dosing for the brain microdialysis method. To overcome this challenge, we propose to dose the cassette compounds in suspension in a simple aqueous formulation in nonintravenous administration routes. In the present study, a cassette of diverse compounds (both in terms of structure and physiochemical properties; summarized in Supplemental Table 1) was dosed as a suspension to rats, either intraperitoneally or subcutaneously, and a dosing regimen was explored that can be

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ABBREVIATIONS: $AUC_{0–360,\ ISF}/AUC_{0–360,\ u,\ p}$, brain interstitial fluid-to-plasma area under the curve ratio from 0 to 360 minutes; BBB, blood-brain barrier; $C_{ss,\ ISF}/C_{ss,\ u,\ p}$, brain interstital fluid-to-plasma concentration ratio at steady state; ISF, Interstitial fluid; PK, pharmacokinetic.

TABLE 1

Plasma protein binding and in vitro recovery of the cassette of compounds used in microdialysis studies

Compound	$f_{u,\ p}$	In Vitro Probe Recovery
Citalopram[a]	0.81 ± 0.20	0.264 ± 0.017
Carbamazepine[a]	0.27 ± 0.02	0.288 ± 0.013
Desmethylclozapine[a]	0.061 ± 0.004	0.320 ± 0.018
Diphenhydramine[a]	0.47 ± 0.04	0.272 ± 0.018
Gabapentin[a]	0.76 ± 0.05	0.225 ± 0.042
Metaclopramide[a]	0.50 ± 0.03	0.237 ± 0.019
Naltrexone[a]	0.53 ± 0.05	0.242 ± 0.015
Quinidine[b]	0.265 ± 0.02	0.294 ± 0.061
Risperidone[b]	0.080 ± 0.005	0.183 ± 0.041

[a]Deshmukh et al. (2015).
[b]Liu et al. (2009).

used to attain steady-state conditions. This allowed for rigorous quantitative assessment of blood-brain partitioning via microdialysis, making microdialysis a more efficient and suitable tool for screening compounds of interest for BBB penetration in a drug discovery setting.

Materials and Methods

Animals and Surgery. For the pharmacokinetic (PK) study, male Sprague-Dawley rats (n = 3 rats per dosing arm) with cannulae implanted in the femoral artery were obtained from Charles River Laboratories, Inc. (Wilmington, MA). For the microdialysis studies, femoral artery–cannulated male Sprague-Dawley rats (250–350 g, 8 to 9 weeks old) with surgically implanted microdialysis guide cannulae (CMA/12; CMA Microdialysis Kista, Sweden), were purchased from Charles River Laboratories, Inc. The guide cannulae had been implanted in the prefrontal cortex at 3.2 mm anteroposterior, 1.0 mm mediolateral, and 0.5 mm dorsoventral to the bregma and secured to the skull with screws and dental cement. Rats were acclimatized to the laboratory environment for 3–5 days before the study.

PK Study. A nine-compound cassette consisting of carbamazepine, citalopram, desmethylclozapine, diphenhydramine, gabapentin, metoclopramide, naltrexone, quinidine, and risperidone was administered via subcutaneous (1 or 2 mg/kg) or intraperitoneal (2 mg/kg) injection in 1% methylcellulose, as a suspension. The dosing volume for subcutaneous administration was 2 ml/kg and the dosing volume for intraperitoneal administration was 5 ml/kg. Plasma samples were taken via the femoral artery cannula at 2, 3, 15, 30, 60, 120, 240, and 480 minutes after dosing. Blood was then centrifuged at 3200g for 5 minutes at 4°C to obtain plasma.

Microdialysis Studies. The principles of a microdialysis study are outlined in Durk (2018). In vitro recovery values of individual compounds for microdialysis

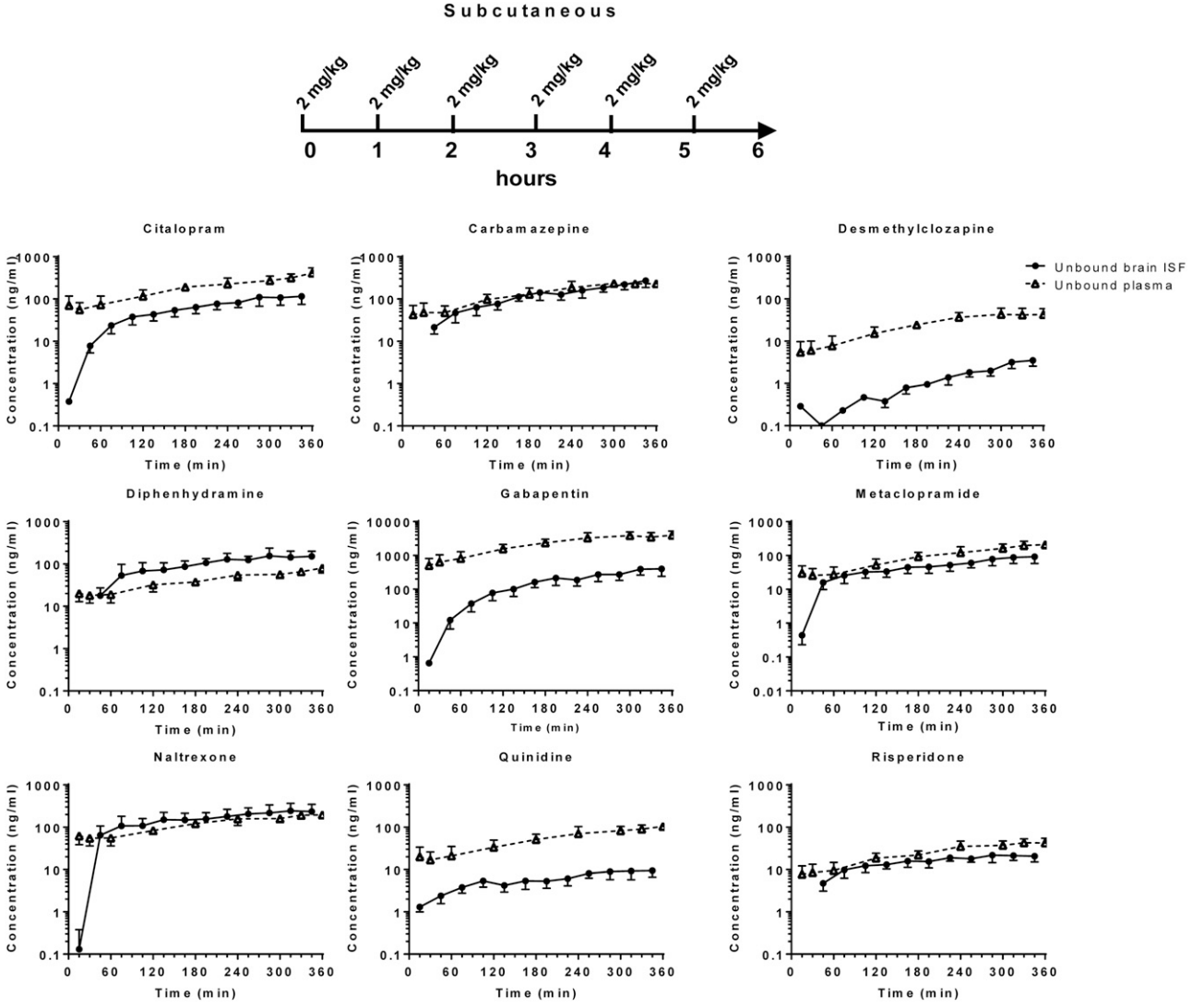

Fig. 1. Unbound drug concentrations in plasma (dotted) and brain ISF (solid, calculated from recovery and dialysate concentration) following repeated subcutaneous administration in rats, every hour. Data are mean ± S.D., n = 4.

TABLE 2

Comparison of brain penetration between intravenous infusion to steady state from Liu et al. (2009) and Deshmukh et al. (2015) and the three dosing regimens employed in the current study

Results in which the dialysate levels were below the limit of quantitation were not calculated.

Compound	$AUC_{0-360,\ ISF}/AUC_{0-360,\ u,\ p}$				$C_{ss,\ ISF}/C_{ss,\ u,\ p}$			
	Intravenous Infusion (n = 3 or 4)	Subcutaneous Dosing		Intraperitoneal Dosing Every 2 Hours (n = 4)	Intravenous Infusion (n = 3 or 4)	Subcutaneous Dosing		Intraperitoneal Dosing Every 2 Hours (n = 4)
		Every Hour (n = 4)	Every 2 Hours (n = 3)			Every Hour (n = 4)	Every 2 Hours (n = 3)	
Citalopram[a]	0.438 ± 0.131	0.293 ± 0.058	0.450 ± 0.284	0.467 ± 0.156	0.400 ± 0.145	0.310 ± 0.0912	0.569 ± 0.351	0.480 ± 0.139
Carbamazepine[a]	0.250 ± 0.0722	0.773 ± 0.0622[b]	0.779 ± 0.265[b]	1.31 ± 0.203[b]	0.249 ± 0.0532	0.860 ± 0.122[b]	0.913 ± 0.345[b]	1.37 ± 0.179[b]
Desmethylclozapine[a]	0.0902 ± 0.0673	0.0466 ± 0.0118	NC	0.0987 ± 0.0452	0.113 ± 0.040	0.0641 ± 0.0261	NC	0.138 ± 0.0716
Diphenhydramine[a]	2.24 ± 0.437	2.06 ± 0.241	2.43 ± 0.957	3.38 ± 0.947	0.679 ± 0.191	2.16 ± 0.529	3.34 ± 1.33[b]	4.18 ± 1.17[b]
Gabapentin[a]	0.0153 ± 0.00514	0.0698 ± 0.0348[b]	0.0622 ± 0.0108	0.0747 ± 0.0230[b]	0.0155 ± 0.00481	0.0846 ± 0.0455[b]	0.0799 ± 0.0185[b]	0.0841 ± 0.0307[b]
Metoclopramide[a]	0.0905 ± 0.0200	0.436 ± 0.0960	0.646 ± 0.317[b]	0.747 ± 0.130[b]	0.0875 ± 0.0201	0.411 ± 0.0981	0.704 ± 0.380[b]	0.803 ± 0.176[b]
Naltrexone[a]	0.441 ± 0.106	1.54 ± 0.267	1.55 ± 0.679[b]	2.78 ± 0.651[b]	0.407 ± 0.100	1.18 ± 0.309	1.88 ± 0.906[b]	3.55 ± 0.601[b]
Quinidine[c]	0.154[d]	0.105 ± 0.0378[e]	NC	0.143 ± 0.0528[e]	0.173 ± 0.109	0.0964 ± 0.0370	NC	0.146 ± 0.0685
Risperidone[c]	0.620[d]	0.549 ± 0.0850[e]	0.776 ± 0.645[e]	0.793 ± 0.204[e]	0.530 ± 0.117	0.520 ± 0.118	0.790 ± 0.653	0.696 ± 0.0938

NC, not calculated.
[a]Deshmukh et al. (2015), n = 4.
[b]$P < 0.05$ between intravenous infusion to steady state and the indicated extravascular dosing group.
[c]Liu et al. (2009), n = 3.
[d]S.D. is not reported because individual animal data were not available for the area under the curve ratio from Liu et al. (2009).
[e]One-way analysis of variance was not performed because individual animal data were not available for the area under the curve ratio from Liu et al. (2009).

probes were determined in a previous study (Deshmukh et al., 2015) and are given in Table 1. Approximately 16 hours before dosing, the rats were placed into individual BASi RATURN systems (Bioanalytical Systems, Inc., West Lafayette, IN) with access to food and water ad libitum. Dummy probes were replaced with CMA 12/2-mm probes (CMA Microdialysis) and perfused with artificial cerebrospinal fluid (CMA Microdialysis) at a rate of 1 μl/min overnight using microdialysis pumps (CMA/102; CMA Microdialysis). On the day of the study, the outlets were connected to BASi Refrigerated HoneyComb Fraction Collectors (Bioanalytical Systems, Inc.) at 4°C and perfused at 1 μl/min. Rats (n = 4) received either a subcutaneous or intraperitoneal dose of a cassette of compounds, consisting of 1 or 2 mg/kg of citalopram, carbamazepine, desmethylclozapine, diphenhydramine, gabapentin, metoclopramide, naltrexone, quinidine, and risperidone. The formulation vehicle was 1% methylcellulose in phosphate-buffered saline, pH 7.4, and the compounds were administered as a suspension at a volume of 2 ml/kg. Three dosing regimens were tested: subcutaneous dosing every hour, subcutaneous dosing every 2 hours, and intraperitoneal dosing every 2 hours. Intraperitoneal dosing every hour was not used because dosing every 2 hours was the most frequent dosing regimen approved by the Genentech Institutional Animal Care and Use Committee. For all subcutaneous and intraperitoneal repeat dose studies, site of injection was rotated between doses, as outlined in the Good Practice Guide to the Administration of Substances and Removal of Blood (Diehl et al., 2001). For all three studies, blood samples were collected via femoral artery cannula 15, 30, 60, 120, 180, 240, 300, 330, and 360 minutes after the first dose. Blood was then centrifuged at 3200*g* for 5 minutes at 4°C to obtain plasma. The perfusate samples were serially collected from each animal for 30-minute intervals from −15 minutes predose to 6 hours postdose. The concentrations are reported at the midpoint of each interval. At 6 hours, the animals were euthanized by intravenous injection of Euthasol (solution of 750 mg/kg phenobarbital and 95 mg/kg phenytoin sodium). All of the samples were stored at −20°C before analysis. All studies were approved by the Genentech Institutional Animal Care and Use Committee.

Liquid Chromatography–Mass Spectrometry. Standard curves and quality control samples were prepared by spiking a known amount of a mixture of the nine compounds into a blank mixed matrix of rat plasma or artificial cerebrospinal fluid. A total of 25 μl of samples, 25 μl of calibration standards, or 25 μl of quality controls were mixed with 5 μl of internal standard (d3-naltrexone) and 200 μl acetonitrile. Following vortexing and centrifugation at 1500*g* for 10–15 minutes, 100 μl of supernatant was transferred to a 96-well plate and diluted with 50 μl water prior to analysis by high-performance liquid chromatography–tandem mass spectrometry.

Data Interpretation. Samples were analyzed using two sets of standard curves and two sets of quality controls in each analytical run. The system consisted of a Shimadzu LC-30 AM pump (Shimadzu, Columbia, MD), and an AB Sciex Qtrap 5000 (AB Sciex, Foster City, CA) mass spectrometer with a turbo ion spray interface. A 20 μl aliquot of each sample was injected into a Kinetex reverse-phase pentafluorophenyl 2.6 μm 100A 50 × 2.1 mm column (Phenomenex, Torrance, CA). The lower limit of quantitation ranged from 0.01 to 0.1 ng/ml. The assay accuracy was between 75% and 125%. Peak quantitation was performed using Analyst Software (SCIEX, Framingham, MA). For the PK study, the PK parameters were determined using Pheonix WinNonlin (Certara, Princeton, NJ). For microdialysis studies, brain penetration was assessed by comparing the brain interstitial fluid (ISF):plasma concentration ratio at steady state ($C_{ss,\ ISF}/C_{ss,\ u,\ p}$) of each compound and the brain ISF:plasma ratio of the area under the curve ($AUC_{0-360,\ ISF}/AUC_{0-360,\ u,\ p}$) throughout the study. Differences between $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ and $AUC_{0-360,\ ISF}/AUC_{0-360,\ u,\ p}$ for extravascular dosing regimens and intravenous infusion to steady state (control dosing regimen) were assessed by one-way analysis of variance and a P value of less than 0.05 was considered to be statistically significant. Differences in the peak-to-trough ratio between the subcutaneous and intraperitoneal dosing regimens were compared using Student's two-tailed t test. A P value of less than 0.05 was considered to be statistically significant.

Results

Single-Dose PK Study. A single-dose cassette of all nine compounds (citalopram, carbamazepine, desmethylclozapine, diphenhydramine, gabapentin, metoclopramide, naltrexone, quinidine, and risperidone), was administered intraperitoneally or subcutaneously. All compounds

were well-absorbed, reached maximum plasma concentration within 1 hour, and were cleared from plasma as time progressed (Supplemental Fig. 1). The parameters for the PK data are summarized in Supplemental Table 2. The PK data were used to confirm that the dosing regimen used would result in estimated dialysate concentrations above the lower limit of quantitation, since $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ was known (using intravenous infusion to steady state) and in vitro probe recovery was also known (Table 1). It was determined that 2 mg/kg would likely yield dialysate concentrations within the limit of quantification. This was confirmed in the microdialysis studies outlined in this paper. Plasma protein binding and in vitro probe recovery values are displayed in Table 1. It is suggested that prior to any microdialysis study with extravascular administration, a single-dose PK study be used to ensure that adequate systemic exposures are attainable.

Repeated Subcutaneous Dose Microdialysis Study, Every Hour. The nine-compound cassette was administered subcutaneously, every hour, during the 6-hour dosing period. In general, the brain:plasma ratio was greater than 1 for diphenhydramine and naltrexone; close to 1 for carbamazepine, metoclopramide, and risperidone; and less than 1 for citalopram, desmethylclozapine, gabapentin, and quinidine (Fig. 1). The $AUC_{0-360,\ ISF}/AUC_{0-360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ values for each dosing regimen are summarized in Table 2. For both $AUC_{0-360,\ ISF}/AUC_{0-360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$, only the ratios for carbamazepine and gabapentin were significantly different from the ratios determined by intravenous infusion to steady state.

Repeated Subcutaneous Dose Microdialysis Study, Every 2 Hours. The nine-compound cassette was administered subcutaneously, every 2 hours, during the 6-hour dosing period. In general, the brain:plasma ratio was close to 1 for diphenhydramine and naltrexone; close to 1 for citalopram, carbamazepine, metoclopramide, and risperidone; and less than 1 for gabapentin (Fig. 2). The $AUC_{0-360,\ ISF}/AUC_{0-360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ values for each dosing regimen are summarized in Table 2. It should be noted that for desmethylclozapine and quinidine, the dialysate concentrations fell below the limit of quantitation, and thus calculation of $AUC_{0-360,\ ISF}/AUC_{0-360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ was not possible. For $AUC_{0-360,\ ISF}/AUC_{0-360,\ u,\ p}$, the values for carbamazepine, metoclopramide, and naltrexone were significantly different from the values determined by intravenous infusion to steady state. For $C_{ss,\ ISF}/C_{ss,\ u,\ p}$, the values for carbamazepine, diphenhydramine, gabapentin, metoclopramide, and naltrexone were significantly different from the values determined by intravenous infusion to steady state.

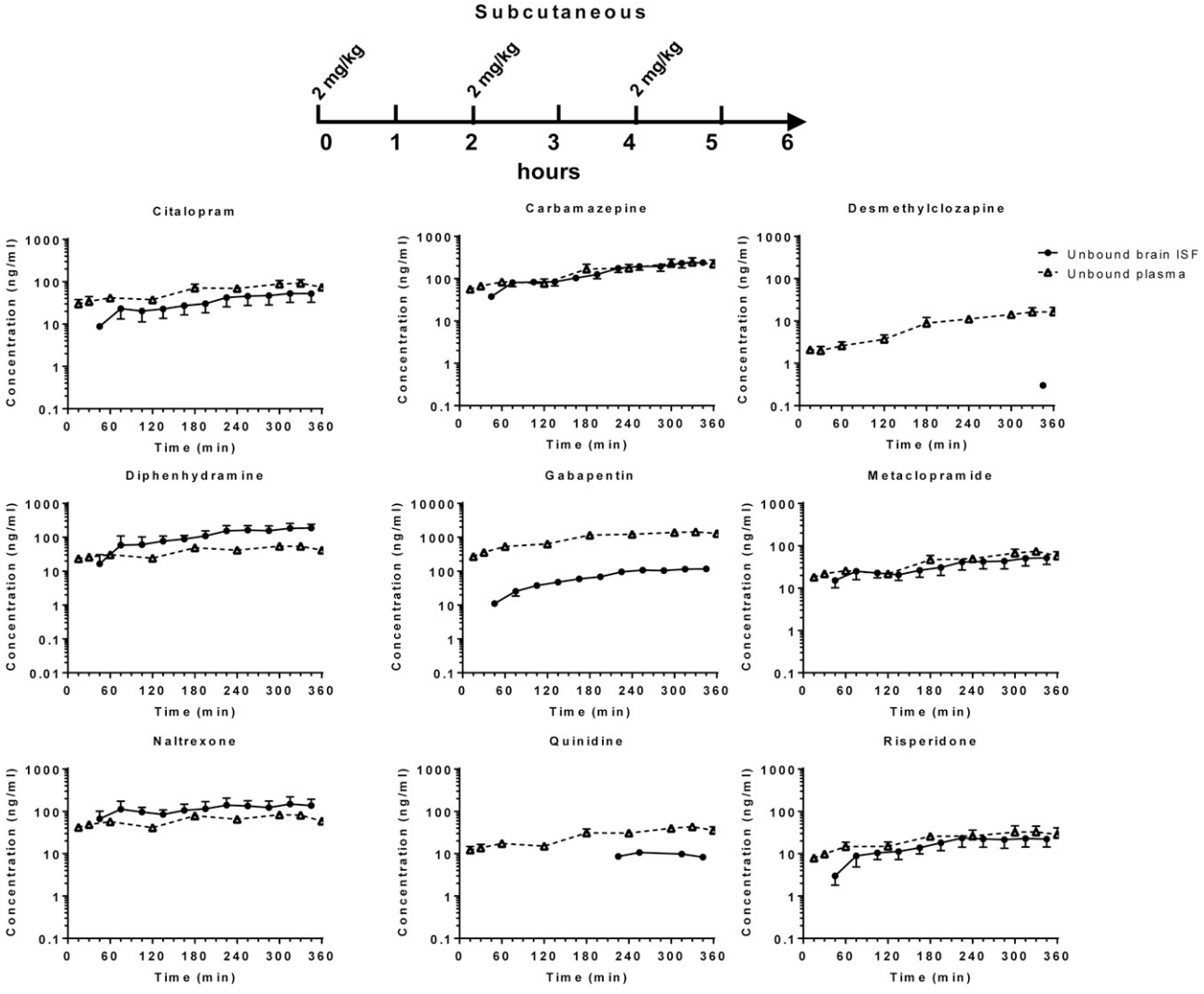

Fig. 2. Unbound drug concentrations in plasma (dotted) and brain ISF (solid, calculated from recovery and dialysate concentration) following repeated subcutaneous administration in rats, every 2 hours. Data are mean ± S.D., $n = 3$. For quinidine and desmethylclozapine, dialysate concentrations were measurable in only one of the three rats and therefore the S.D. values were not reported and brain:plasma ratios were not calculated.

Repeated Intraperitoneal Dose Microdialysis Study. The nine-compound cassette was administered intraperitoneally, every 2 hours, during the 6-hour dosing period. In general, the brain:plasma ratio was greater than 1 for diphenhydramine and naltrexone; close to 1 for carbamazepine, metoclopramide, and risperidone; and less than 1 for citalopram, desmethylclozapine, gabapentin, and quinidine (Fig. 3). The $AUC_{0–360,\ ISF}/AUC_{0–360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ values for each dosing regimen are summarized in Table 2. For $AUC_{0–360,\ ISF}/AUC_{0–360,\ u,\ p}$, the values for carbamazepine, gabapentin, metoclopramide, and naltrexone were significantly different from the values determined by intravenous infusion to steady state. For $C_{ss,\ ISF}/C_{ss,\ u,\ p}$, the values for carbamazepine, diphenhydramine, metoclopramide, and naltrexone were significantly different from the values determined by intravenous infusion to steady state.

Discussion

The results of this study demonstrate that repeated extravascular dosing of a cassette in suspension can be used in place of an intravenous cassette infused to steady state when screening compounds in brain microdialysis studies. This is a significant improvement in avoiding extensive formulation work that may be required to deliver a diverse cassette of compounds as an intravenous dose in solution. In addition, further steps may be taken to reuse rats with microdialysis probes implanted; a number of studies have examined to what extent rats with implanted microdialysis probes may be reused (de Lange et al., 1994), but this appears to be dependent on the probe compound and also that the study be performed under carefully controlled conditions. A different approach was taken by Durk et al. (2015), in which probes were implanted in each side of the brain and the same set of rats was used twice, also saving considerable time and resources.

The set of compounds used in the present study was chosen because a cassette microdialysis data set was already available following intravenous infusion (Deshmukh et al., 2015), and these compounds are readily available to any researcher and can be used as internal standards for future studies, administered with unknown compounds to benchmark the extent of brain penetration. In addition to the

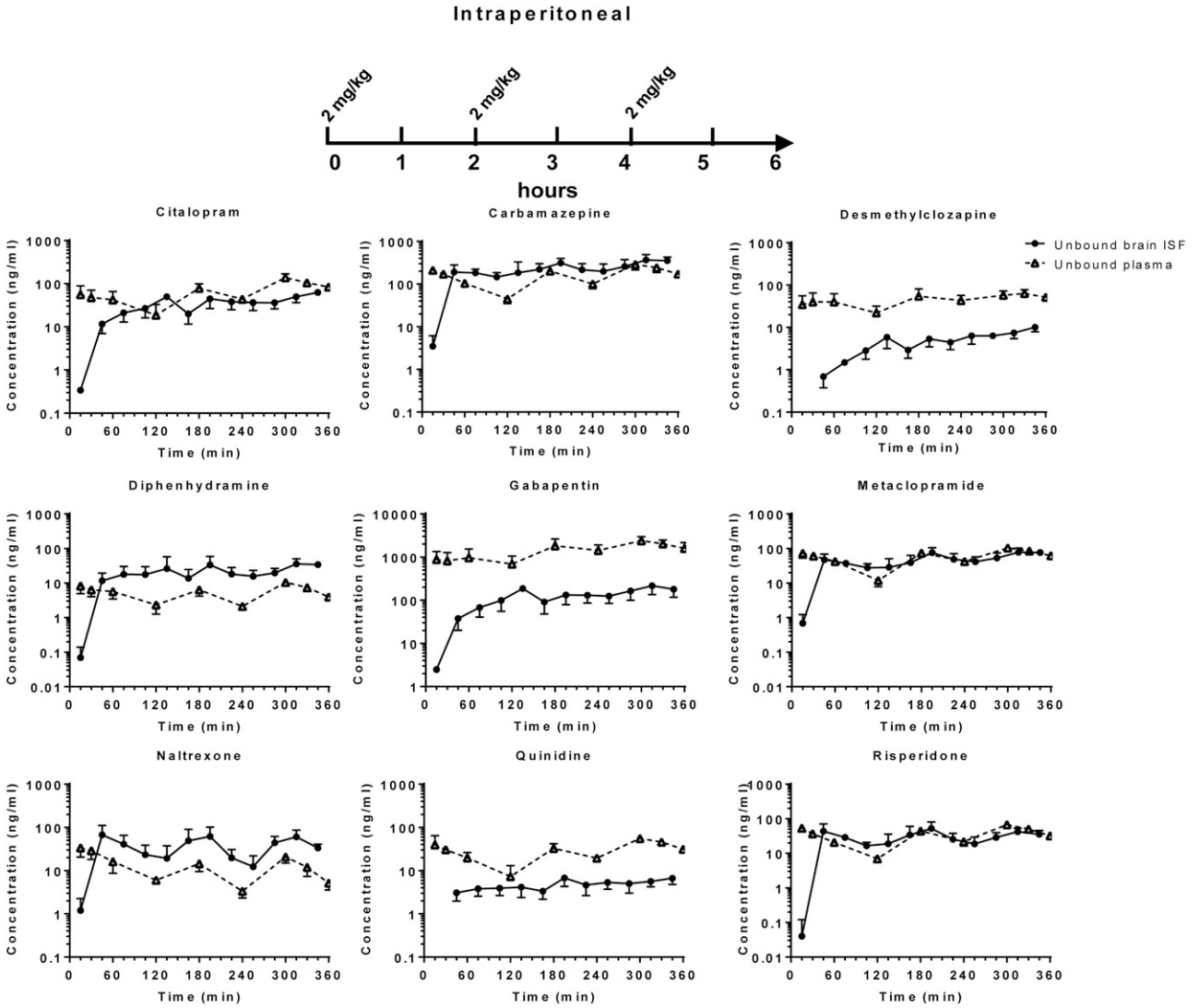

Fig. 3. Unbound drug concentrations in plasma (dotted) and brain ISF (solid, calculated from recovery and dialysate concentration) following repeated intraperitoneal administration in rats, every 2 hours. Data are mean ± S.D., $n = 4$.

compounds selected from Deshmukh et al. (2015), two additional compounds from Liu et al. (2009), quinidine and risperidone, were added to better represent P-gp substrates.

It should be noted that all three dosing regimens, in addition to intravenous infusion to steady state, consistently allow for the categorization of compounds as net efflux (brain-to-plasma unbound tissue partition coefficient $<<$ 1), passive distribution (brain-to-plasma unbound tissue partition coefficient $\approx$ 1), or net uptake (brain-to-plasma unbound tissue partition coefficient $>>$ 1). While more definitive studies may need to be carried out to quantitatively assess brain concentrations for compounds of interest, the screening paradigm outlined in this study allows for early screening without extensive formulation work that may be required for intravenous dosing.

One further parameter that plays an important role in assessing the brain:plasma ratio in such a short dosing duration is the rate at which tissue distribution occurs. If a compound is lipohilic and nonpolar and the capillary membrane does not act as a physical barrier to the compound, then the rate of distribution will be dependent on the vascular blood flow to that tissue. However, the presence of tight junctions at the BBB will limit the rate of absorption such that the rate-determining step will be permeation across the capillary membrane, which is related to molecular weight, charge, and lipophilicity (Goresky et al., 1970; Liu et al., 2005). Because of this, large, charged, and more polar compounds will take longer to distribute to tissues, and this was observed, as an example, for zwitterionic gabapentin versus lipophilic, weakly basic diphenhydramine.

Generally speaking, for all compounds in the nine-compound cassette, subcutaneous dosing resulted in $AUC_{0–360,\ ISF}/AUC_{0–360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ ratios that were closest to those obtained from intravenous infusion dosing, whereas intraperitoneal dosing resulted in ratios that were less accurate. The fewest significant differences were observed between intravenous infusion to steady state and the every hour subcutaneous dosing regimen compared with the other regimens, when $AUC_{0–360,\ ISF}/AUC_{0–360,\ u,\ p}$ and $C_{ss,\ ISF}/C_{ss,\ u,\ p}$ values were compared between dosing regimens using one-way analysis of variance. One possible explanation for this observation is that intraperitoneal dosing undergoes faster absorption to the systemic circulation versus a subcutaneous dose, and this is less similar to steady-state intravenous infusion than subcutaneous dosing. This is confirmed by comparing the peak-to-trough ratio for each dosing regimen. Hourly subcutaneous dosing yielded peak-to-trough ratios of 1 or less. Statistical comparison of subcutaneous and intraperitoneal dosing, administered every 2 hours, showed that for all compounds except citalopram, the peak-to-trough ratio was significantly higher with intraperitoneal versus subcutaneous dosing (Supplemental Table 3). Since systemic clearance is the same no matter what route of administration is used, it is likely that apparent clearance is determined by rate of absorption, and this rate appears to be faster with intraperitoneal versus subcutaneous dosing. Further studies may be needed to optimize the dosing regimens for individual compounds, but the every hour dosing regimen is a good starting point that works well for most compounds. This approach also minimized the chances of a sample being below the limit of quantification, especially in the dialysate, in which concentrations may be near or below the limit of detection due to low recovery, low brain penetration, or a combination thereof.

Despite these differences in physiochemical properties and time to reach steady state, repeated extravascular dosing was shown to be a suitable alternative to intravenous infusion to steady state in cassette microdialysis studies to determine brain penetration of a diverse set of compounds.

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Contributed new reagents or analytic tools: Durk, Ding.
Performed data analysis: Durk, Deshmukh, Ding.
Wrote or contributed to the writing of the manuscript: Durk, Liu.

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