The Design and Validation of a Novel Intravenous Microdialysis Probe: Application to Fluconazole Pharmacokinetics in the Freely-Moving Rat Model

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Purpose. The purpose of this study was to design and validate a concentric, flexible intravenous microdialysis probe to determine drug concentrations in blood from the inferior vena cava of a freely-moving animal model.

Methods. An intravenous microdialysis probe was constructed using fused-silica tubing and an acrylonitrile/sodium methallyl sulfonate copolymer hollow fiber. The probe was tested *in vitro* for the recovery of fluconazole and UK-54,373, a fluconazole analog used for probe calibration by retrodialysis. Subsequent *in vivo* validation was done in rats (n = 7) that had a microdialysis probe inserted into the inferior vena cava via the femoral vein, and the femoral artery was cannulated for simultaneous blood sampling. Comparisons of fluconazole pharmacokinetic parameters resulting from the two sampling methods were performed at 2 and 10 days after probe implantation.

Results. There were no statistical differences between the microdialysis sampling and conventional blood sampling methods for the $T_{1/2}$, Cl, Vdss, and dose-normalized AUC by paired t-test ($p > 0.05$) for repeated dosing at day 2 and day 10 after probe placement. The probe recovery, as determined by retrodialysis, significantly decreased over the ten day period. This finding indicates the necessity for frequent recovery determinations during a long-term blood microdialysis experiment.

Conclusions. These results show that microdialysis sampling in the inferior vena cava using this unique and robust probe design provides an accurate method of determining blood pharmacokinetics in the freely-moving rat for extended experimental periods. The probe design allows for a simple surgical placement into the inferior vena cava which results in a more stable animal preparation for long-term sampling and repeated-measures experimental designs.

KEY WORDS: intravenous microdialysis; blood sampling; fluconazole; pharmacokinetics.

INTRODUCTION

When compared to traditional blood sampling methods, blood microdialysis coupled with on-line HPLC analysis provides a powerful tool to continuously monitor the extracellular free drug concentration in the blood of animals for metabolic and pharmacokinetic purposes (1,2). Advantages (3,4) of this technique include: 1) studies may be done in freely-moving, conscious animals, 2) frequent determinations may be made, which can provide more information about the shape of the drug concentration-time profile and allow the use of the same animal for multiple experiments, without concern for blood loss from small animals, 3) continuous sampling for long periods of time without altering the pharmacokinetics due to physiological changes that result from blood sampling is possible, and 4) *in vivo* determination of unbound drug concentration in the blood can be performed.

Several reports have recently been published describing the application of intravenous microdialysis sampling to the study of the pharmacokinetics of drugs in laboratory animals, mainly in the rat. Most intravenous microdialysis probes currently used (5,6) are designed to be most easily placed in the jugular vein of the rat. However, for long-term use in a freely-moving animal, the placement of the microdialysis probe in the jugular vein may not be secure. In addition, the convective flow of blood around the probe implanted in the jugular vein may be small and variable thus resulting in fluctuations in the probe recovery (7).

We now report a concentric, flexible microdialysis probe, which is placed in the inferior vena cava through the femoral vein and allows frequent blood sampling for a prolonged period of time in freely-moving rats. The performance of this blood microdialysis probe was examined *in vitro* and *in vivo* and compared with traditional blood sampling for determining the concentration of fluconazole in blood extracellular fluid. Fluconazole was chosen as the model drug for this study because we are currently studying fluconazole disposition in the central nervous system (CNS) using microdialysis, and our repeated crossover study design for fluconazole CNS distribution would be limited by excessive blood withdrawal. Our current results show that the intravenous microdialysis sampling in the inferior vena cava using this probe design provides an accurate method of determining the blood pharmacokinetics of fluconazole in the freely-moving rat for up to 10 days. When the probe recovery is appropriately determined, this microdialysis probe design may be utilized to sample the blood concentration of compounds that are suitable for microdialysis for extended pharmacokinetic studies, including repeated dosing experiments, in small animals. Our experience with determining fluconazole pharmacokinetics using microdialysis sampling in the inferior vena cava is similar to an elegant study recently reported in this journal by Evrard *et al.* (7). In that study, the authors report using a different probe design (linear probe) to examine the pharmacokinetics of flubiprofen in a short term single-dose experimental design (7).

MATERIALS AND METHODS

Microdialysis Probe Manufacture

Figure 1 shows a diagram of the concentric, flexible blood microdialysis probe. A 1-meter-long (98-μm I.D., 165-μm O.D.) fused-silica tubing (Polymicro Technologies, Inc., Arizona) was used as the inlet, and a 1-meter-long (145-μm I.D., 210-μm O.D.) fused-silica tubing was used as the outlet. A commercially available hemodialyzer hollow fiber (Filtral 20, AN69, acrylonitrile/sodium methallyl sulfonate copolymer, I.D. 220 μm, O.D. 310 μm, Hospal, Cobe Renal Care, Lakewood, CO, 80215) with a MWCO of 40,000 to 42,000 daltons, was used as the semipermeable microdialysis membrane. Two 1.5-cm-long 22-gauge catheters (I.D. 420 μm, O.D.670 μm) were

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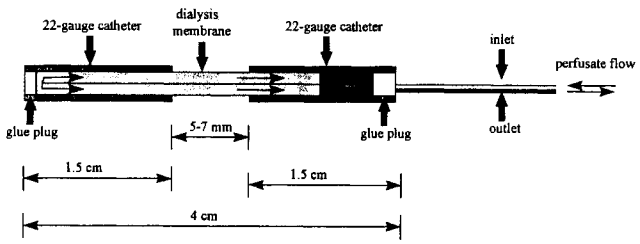

Fig. 1. The concentric, flexible intravenous microdialysis probe.

cut from an intravenous placement unit (I-CATH®, Charter Med, Inc., Lakewood, NJ 08701) and were used to secure the probe membrane to the fused-silica inlet and outlet tubing. Briefly, one end of a 22-gauge catheter was sealed with cyanoacrylate glue at least two days before probe manufacture to allow complete drying of the glue. These catheters were then used as the probe tip. A 2-cm-long length of the hemodialyzer hollow fiber was then inserted into the 22-gauge catheter probe tip. The other end of the hemodialyzer hollow fiber was then partially inserted into the second 22-gauge catheter, allowing a 5–7 mm long active surface for solute exchange. The junctions between the hemodialyzer hollow fiber and the catheters were sealed with cyanoacrylate glue, carefully avoiding any glue spreading onto the exposed surface area of the membrane, and were allowed to dry for approximately 30 minutes. The inlet fused-silica tubing was then inserted into the open end of the second catheter, and through the lumen of the hemodialyzer hollow fiber into the catheter at the probe tip, extending to within 0.5–1 mm of the sealed end of the tip. The outlet fused-silica tubing was also inserted into the open end of the second catheter, and did not extend to the hemodialyzer hollow dialysis fiber (see Figure 1). Finally, the open end of the second catheter was sealed to both the inlet and outlet fused silica tubings using cyanoacrylate glue, with care taken to avoid glue spreading to the tip of the outlet tubing. Intravenous microdialysis probes were allowed to cure at room temperature for at least one day before surgical placement. Some probes were constructed at least one month prior to use, indicating that probes can be manufactured in a batch and stored for extended time periods when kept dry.

This method of probe manufacture results in a flexible probe tip that is very robust. The outlet tubing has a greater interior diameter than the inlet tubing, to reduce the pressure gradient across the dialysis membrane, thereby eliminating fluid flux out of the probe while maintaining a one meter outlet length. Moreover, the placement of the catheter segment at the tip of the probe makes the surgical insertion of the probe into the inferior vena cava via the femoral vein a procedure that can be performed with relative ease, similar to vessel cannulation using a simple catheter.

Animal Studies

Seven male Sprague-Dawley rats weighing between 250–350 g were used in this study. At all times, including the microdialysis sampling period, the rats had free access to food and water. Surgical preparation of these rats was done using aseptic technique, followed by a recovery period of at least 12 hours. All surgical procedures were performed under anesthesia using an intraperitoneal dose of 50 mg/kg sodium pentobarbital. An intramuscular dose of 60,000 units procaine penicillin G was given following surgery. The vascular cannula were made with a PE-50 tubing, which was connected to a 5 to 6-cm length of PE-10 tubing using cyanoacrylate glue.

A small cut (0.5 to 1 cm) was made in the skin of the rat at the back of the neck, and a subcutaneous tunnel was made from this site to the inguinal area. All catheters and probe lines route subcutaneously from the insertion point at the femoral site to a spring tether system at the back of the neck. This system protected the lines from the rat and allowed the animal free movement throughout the experimental period. The femoral artery and vein were surgically exposed and separated from surrounding tissues. The isolated vein was tied off at the distal end and a small transverse nick was made in the vein using a straight microdissecting spring scissors. For rats 1 to 4, the microdialysis probe was inserted 4.0 cm into the vessel, and the fluconazole dose was given via the arterial cannula. For rats 5 to 7, a microdialysis probe together with a venous cannula were inserted into the inferior vena cava via the femoral vein, and the fluconazole doses were given via the venous cannula. The artery was constricted at the proximal end using gentle pressure applied by pulling a loop of 4/0 silk suture, and then a small transverse nick was made in the artery. The PE-10 tubing was then inserted 4.0 cm into the artery for subsequent drug administration and/or blood sampling. To avoid contamination of the arterial samples by the dose, after the administration of the fluconazole dosing solution through the arterial cannula, a 0.5 ml 40 unit/ml heparinized saline solution was pushed through the arterial cannula, followed by drawing 0.3 ml blood and then pushing it back to the rat three times using a clean syringe. Finally, a 40 unit/ml heparinized saline solution was maintained in the arterial cannula to prevent blood clotting.

Fluconazole doses of 10 mg/kg and 20 mg/kg were administered by intra-arterial (rats 1–4) or intravenous (rats 5–7) bolus to the rats. Rats #1 and #7 received 20 mg/kg fluconazole on day 2 and day 10 after probe placement, and rats #2 and #5 received 10 mg/kg fluconazole on day 2 and day 10 after probe placement. Rat #3 received a 10 mg/kg dose, and rats #4 and #6 received 20 mg/kg fluconazole dose only on day 2 after the cannulation. This was due to clotting problems in the arterial cannula on day 10 after the cannulation.

In vivo probe recovery was determined by using UK-54,373, an analog of fluconazole, as a retrodialysis calibrator (10). A 0.3 ml blood sample was obtained at different time points after dosing, and the plasma was harvested and stored frozen at −20°C until analysis.

Sample Analysis

Fluconazole concentrations in the plasma, ultrafiltrate, and microdialysate samples were determined by HPLC with UV detection, according to the method of Flores-Murrieta *et al.* (8) with some modifications.

Microdialysis samples from the blood were collected on-line directly into 10-μl HPLC-auto-injection loops over a collection interval of 20 minutes. The peak heights of fluconazole and UK-54,373 that resulted from a single loop fill using syringe pump perfusion (0.5 μl/min × 20 minute, and a 10 μl-loop) and the multiple loop fill (approximately 150 μl) by manual injection of the standard fluconazole solution were not statistically different. This is an important consideration for on-line

analyses of microdialysates in generating an accurate standard curve for quantitation (20,21). Therefore, the concentration-response calibration curve was determined following each experiment, by directly injecting about 150 μl of fluconazole standard solution into the auto-injection loop and measuring fluconazole peak height.

Determination of Unbound Fraction of Fluconazole in Rat Plasma

Ultrafiltration was performed with a Centrifree micropartition system (Amicon, Beverly, MA 01915). Blank plasma was spiked with fluconazole to give fluconazole concentrations of 0.5, 8, 20, and 40 μg/ml, which covered the observed concentration range in the *in vivo* experiments.

Determination of Microdialysis Recovery by Gain and Loss

The microdialysis probe recoveries by gain (RG) and by loss (RL) *in vitro* were determined using methods previously described (10). Microdialysis probe recovery *in vivo* was estimated in each animal by retrodialysis during all microdialysis procedures, utilizing UK-54,373 as a retrodialysis calibrator (10).

Determining Fluconazole Concentration in Plasma by Microdialysis

In our animal experiments, the probe recovery was monitored continuously throughout the microdialysis experiment (i.e., probe recovery was determined for every 20 minute collection interval), and the unbound fluconazole concentrations in the blood (Ci) were calculated using equation 1:

$$Ci = Cout_i/RL_i \quad (1)$$

where Ci is the calculated unbound fluconazole concentration, $Cout_i$ is fluconazole concentration in the dialysate for ith collection, and RL_i is the probe recovery for ith collection determined using UK-54,373 retrodialysis and recovery by loss (10).

Pharmacokinetic Analysis

Fluconazole concentrations determined by intravenous microdialysis were corrected for the unbound fraction of fluconazole in rat plasma to give the plasma total fluconazole concentration. The plasma total fluconazole concentration-time profiles following fluconazole bolus dosing were described by a one compartment, first-order elimination model. Nonlinear least-squares regression analysis was performed using the PCNONLIN program (SCI Software Inc. Lexington, KY 40504). The area under the plasma concentration versus time curve (AUC) and area under the first moment curve (AUMC) were also calculated by PCNONLIN. The mean residence time (MRT) was determined as the ratio of AUMC/AUC. The systemic plasma clearance (CL) was calculated from the ratio of dose/AUC. The volume of distribution at steady state (Vdss) was obtained from the product of CL*MRT. For comparison purposes, the AUC was also determined using linear trapezoidal and log-linear trapezoidal numerical integration methods (12). Finally, a paired t-test was performed for each of the pharmacokinetic parameters to compare the parameters determined using intravenous microdialysis sampling with those parameters determined using traditional blood sampling.

RESULTS

Comparison of Relative Recovery of Fluconazole and UK-54,373 *In Vitro*

The comparison of the relative probe recovery between fluconazole and UK-54,373 has been performed *in vitro* from buffer using five different intravenous microdialysis probes (Table I). The mean ± S.D. relative recoveries of fluconazole and UK-54,373 for recovery by gain (RG) and recovery by loss (RL) are listed in Table I. There was no statistical difference between the RG and RL for either compound. The RG of fluconazole was not statistically different from the RL of UK-54,373.

Unbound Fraction of Fluconazole in Rat Plasma

The average unbound fraction of fluconazole in rat plasma was 0.92 ± 0.04, with a range of 0.88 to 0.96 for the four concentrations studied. There were no concentration-dependent changes in the unbound fraction of fluconazole within the concentration range studied. The unbound fraction of fluconazole in rat plasma determined in this study was similar to the previously reported value of 0.91 (9).

Table I. Comparison of the Relative Recovery Between Fluconazole and UK-54,373 *In Vitro*[a]

Probe	RG[b] (fluconazole)	RL[c] (fluconazole)	RG[b] (UK-54,373)	RL[c] (UK-54,373)
1	0.68[d]	0.67	0.68	0.75
2	0.59	0.70	0.57	0.63
3	0.67	0.69	0.69	0.69
4	0.65	0.59	0.67	0.61
5	0.76	0.78	0.76	0.77
Mean ± S.D.	0.67 ± 0.06	0.69 ± 0.07	0.67 ± 0.07	0.69 ± 0.07

[a] Intravenous microdialysis probes, perfusion rate: 0.5 μl/min, 37°C.
[b] Recovery by gain.
[c] Recovery by loss.
[d] Recovery listed as the mean of several collection intervals for each probe.

Table II. Intravenous Microdialysis Probe Recovery *In Vivo*

Rat	RL[a] (day 2)	RL[a] (day 10)
1	0.38 ± 0.05 (n = 58)[c]	0.26 ± 0.04 (n = 29)
2	0.45 ± 0.07 (n = 40)	0.34 ± 0.04 (n = 29)
3	0.46 ± 0.05 (n = 36)	ND[d]
4	0.48 ± 0.04 (n = 37)	ND
5	0.81 ± 0.03 (n = 62)	0.68 ± 0.03 (n = 70)
6	0.63 ± 0.04 (n = 74)	0.45 ± 0.04 (n = 65)
7	0.44 ± 0.02 (n = 83)	0.35 ± 0.03 (n = 64)
Mean ± S.D.	0.52 ± 0.15[b]	0.42 ± 0.16[b]

[a] Recovery by loss using the retrodialysis of UK-54,373.
[b] There is a statistical difference in the probe recovery between day 2 and day 10. (paired t-test, $p < 0.05$).
[c] Number of separate in vivo collection intervals.
[d] Experiments were not performed because of the arterial cannula clotting.

Intravenous Microdialysis Probe Recovery *In Vivo*

The mean ± S.D. relative recoveries by loss of UK-54,373 in blood at day 2 and day 10 after probe placement are listed in Table II. The probe recovery in blood was significantly lower than that in buffer, and declined gradually with time (Figure 2). The average probe recovery at day 2 was significantly higher than that at day 10 after probe placement.

In Vivo Comparison of Blood Microdialysis Sampling versus Traditional Blood Sampling

The fluconazole plasma concentration-time profiles determined using intravenous microdialysis, after being corrected for the unbound fraction, were similar to those determined using traditional blood sampling for both doses in all seven rats. Moreover, similar concentration-time profiles for these two methods were obtained from experiments done on day 2 and day 10 after probe placement (Figure 3). Pharmacokinetic parameters determined from the concentration-time data derived from each sampling method are listed in Table III. They are similar to previous reports (10,11). There was no statistical difference in any pharmacokinetic parameter between these two sampling methods for day 2 and day 10 after probe placement,

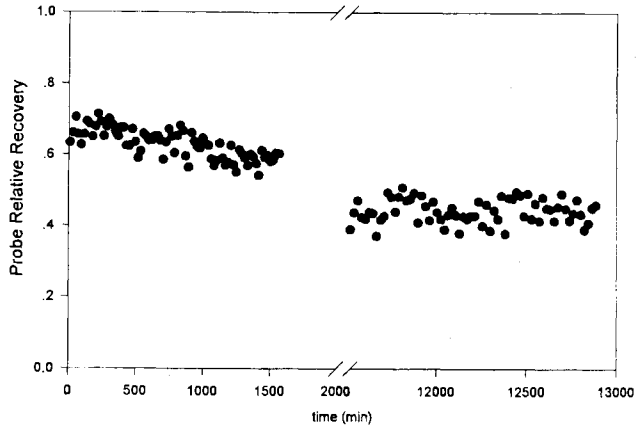

Fig. 2. The time course of the relative microdialysis probe recovery *in vivo* from blood for a representative rat. The probe recovery was determined by recovery by loss using the retrodialysis of UK-54,373.

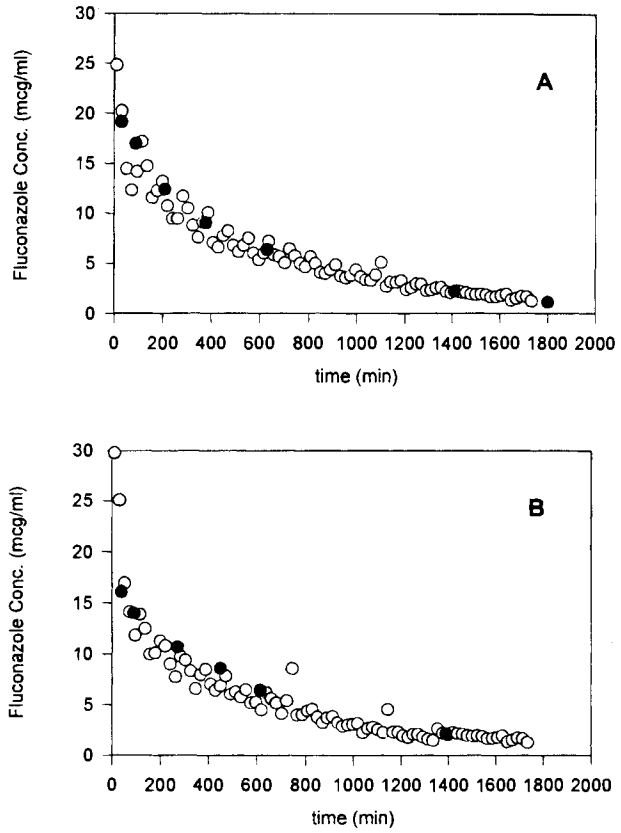

Fig. 3. Fluconazole total concentration-time profile for a representative rat on day 2 after cannulation (A), and on day 10 after cannulation (B). (O) from blood microdialysis sampling; (•) from traditional blood sampling.

as determined using a paired t-test ($p > 0.05$). This result validates the use of the microdialysis technique to determine standard pharmacokinetic parameters after long-term implantation of the probe (10 days).

Comparison of AUCs Estimated by the Linear Trapezoidal, Log Trapezoidal, and PCNONLIN Methods

The AUCs were estimated by PCNONLIN, linear trapezoidal, and log trapezoidal methods (12), and are listed in Table IV. For microdialysis sampling data, all three numerical integration algorithms give similar AUC values. However, for the traditional blood sampling method, the linear trapezoidal method results in a significantly higher AUC value when compared to the other two methods. The AUCs of data derived from traditional blood sampling estimated by the log trapezoidal and PCNONLIN methods are similar to the AUCs of microdialysis data estimated by all three methods.

DISCUSSION

We have developed a concentric, flexible intravenous microdialysis probe to sample the unbound drug concentration in the blood of the rat. All other currently used concentric intravenous microdialysis probes have the dialysis fiber at the tip of the probe, which, as recently pointed out by Evrard *et*

Table III. Fluconazole Pharmacokinetic Parameters Determined Using Intravenous Microdialysis Versus Traditional Blood Sampling

Pharmacokinetic parameters derived from data collected by traditional blood sampling[a]					
		AUC T (mg*min/L)	T1/2 (min)	CL (ml/min/kg)	Vds (L/kg)
day 2	(n = 7)	4523 ± 869	347 ± 78	2.28 ± 0.44	1.11 ± 0.14
day 10	(n = 4)	4329 ± 1239	367 ± 97	2.46 ± 0.71	1.24 ± 0.18
10 mg/kg	(n = 5)	4351 ± 717	338 ± 48	2.35 ± 0.40	1.14 ± 0.21
20 mg/kg	(n = 6)	4537 ± 1190	368 ± 104	2.34 ± 0.65	1.17 ± 0.12
all data	(n = 11)	4452 ± 961	354 ± 81	2.35 ± 0.52	1.15 ± 0.16
Pharmacokinetic parameters derived from data collected by microdialysis sampling[a]					
		AUC (mg*min/L)	T1/2 (min)	Cl (ml/min/kg)	Vdss (L/kg)
day 2	(n = 7)	4559 ± 1200	360 ± 83	2.26 ± 0.50	1.14 ± 0.21
day 10	(n = 4)	4863 ± 944	355 ± 92	2.58 ± 0.84	1.24 ± 0.14
10 mg/kg	(n = 5)	4306 ± 739	351 ± 41	2.37 ± 0.41	1.19 ± 0.16
20 mg/kg	(n = 6)	4973 ± 1277	364 ± 109	2.38 ± 0.80	1.16 ± 0.22
All data	(n = 11)	4669 ± 1075	358 ± 82	2.38 ± 0.62	1.18 ± 0.18

[a] No statistical difference between traditional blood sampling and microdialysis sampling in the derived pharmacokinetic parameters.

al. (7), may result in membrane damage during the implantation procedure. Thus, the most popular probe placement has been in the jugular vein (5–7). In our probe design, a section of a 22-gauge catheter was used as the probe tip, which successfully overcame the drawback of other concentric probe designs, and allowed the probe to be easily inserted into the inferior vena cava through the femoral vein, much like the insertion of a common catheter. Compared to the linear probe that has been used for sampling drug concentration in inferior vena cava (7), our probe construction is simpler, and in our hands, easier to implant into the inferior vena cava. Given that the probe implantation is similar to a blood vessel cannulation, we are also able to place a probe together with a blood cannula into the inferior vena cava through the same femoral vein. In addition, by using the dialysis fiber with high MWCO (40,000 to 42,000 daltons), we can get approximately 50% recovery at day two and approximately 40% recovery at day ten from the blood, using 5–7 mm dialysis hollow fiber at a 0.5 μl/min perfusion rate. A higher probe recovery may be obtained by increasing the length of the dialysis hollow fiber, however, when using retrodialysis, we prefer a recovery value between 40–60%, to allow for greater precision in determining recovery. This is because as the recovery approaches 100%, the peak response of a retrodialysis calibrator (recovery by loss) approaches zero, increasing the variability of measured peak responses from one interval to another.

Table IV. Area Under the Plasma Concentration Time Curve for Fluconazole Calculated Using Model Fits, Linear Trapezoidal, and Logarithmic Trapezoidal Methods

	Microdialysis data AUC (mg*min/L)	Blood sampling data AUC (mg*min/L)
Linear trapezoidal	4495 ± 1183	4807 ± 1071[a]
Logarithmic trapezoidal	4406 ± 1195	4400 ± 1007
Model fit[b]	4464 ± 1195	4452 ± 961

[a] Significantly greater than other values by paired t-test.
[b] Area under the curve determined from one-compartment model parameter estimates.

It is important to know the relative probe recovery *in vivo* to accurately determine drug concentration in the plasma or tissue surrounding the probe. Several previous studies have indicated that the *in vitro* recovery usually is an overestimate of *in vivo* recovery (10,13–16). This is because the resistance to diffusion (which is a determinate of recovery) is usually less in the *in vitro* media than in the tissue in the *in vivo* situation. It was observed in the present study that for UK-54,373, the *in vivo* recovery by loss is significantly less than the *in vitro* recovery by loss ($p < 0.01$ by student t-test), and this recovery declined with time. This result indicates that *in vivo* probe calibration is necessary for the accurate estimation of drug concentration in the blood. Our previous results show that fluconazole and UK-54,373 have similar dialysis characteristics *in vitro* and *in vivo* (10), and that UK-54,373 may be used as the probe calibrator for estimating the recovery of fluconazole. Furthermore, the recovery has no directional dependence, suggesting that the retrodialysis (recovery by loss) can be directly used to estimate the recovery by gain. Moreover, we found that the *in vivo* probe recovery declined gradually with time, suggesting a decrease in membrane dialysis clearance over time. A similar phenomenon has been observed by others in clinical hemodialysis studies (17,18). This reduction in membrane clearance (recovery) was attributed to fiber clotting and changes in membrane properties due to plasma protein and/or cell deposition on the surface of the membrane. They also found that the magnitude of the reduction in solute clearance depended on the particular solute and the type of dialysis membrane.

Our results indicate that the probe recovery determined before and after the intravenous microdialysis experiment was not an accurate estimation of the probe recovery during individ-

ual intervals in our long-term experiment (i.e., especially when comparing day 2 and day 10). In our experiments, the probe recovery was monitored continuously throughout the microdialysis experiment using on-line retrodialysis, and fluconazole concentrations in the blood (Ci) were calculated using equation 1. Therefore, using continuous monitoring of the recovery allows for the accurate determination the pharmacokinetics of fluconazole in the blood even for extended experimental protocols.

The fluconazole plasma concentration-time profiles and pharmacokinetic parameters determined using intravenous microdialysis, after correction for the fraction unbound, were similar to those determined using traditional blood sampling. This study demonstrates that blood microdialysis is a feasible method to use in sampling the plasma fluconazole concentration for up to 10 days post probe implantation.

The area under the concentration-time curve (AUC) is one of the most important parameters in pharmacokinetic analysis. The value of AUC may be obtained by fitting an analytical function based on a compartment model to experimental concentration versus time data. Alternatively, it may be estimated by direct numerical integration of the data, using a variety of numerical integration algorithms such as the linear and log-trapezoidal methods, the Lagrange method, and the spline method (12). For monoexponentially decaying data, especially when the frequency of blood sampling is limited, the linear trapezoidal method can produce large positive errors (12). In this case, the log-trapezoidal method is preferred when compared with the linear trapezoidal, Lagrange, and spline methods (12,19). The AUC values determined by fitting a monoexponential equation to the data (PCNONLIN), linear trapezoidal, and log-trapezoidal methods for traditional blood sampling and intravenous microdialysis sampling are shown in Table IV. We found that for traditional blood sampling, because of the long intervals between data points, the AUC estimated by linear trapezoidal method is significantly greater than the AUC values obtained from the other two methods. However, for the microdialysis data, the AUC values determined by each of the three methods are similar. These results indicate that the simple linear trapezoidal method may be used to accurately calculate the AUC for microdialysis data, because the sampling intervals can be frequent with respect to the elimination half-life of the drug.

Recently, we have used this intravenous microdialysis probe in a four-way crossover study to investigate the distributional kinetics of fluconazole across the blood-brain barrier. In this study, four different bolus doses of fluconazole were given to the same rat, and the fluconazole concentration in the plasma and brain tissue were simultaneously monitored by microdialysis for 6–7 days. The blood microdialysis probe functioned well during this four-way crossover study, which would have not been possible using other blood sampling techniques. The use of blood microdialysis sampling is generally applicable, and the probe may be used as a sampling tool for other drugs that have sufficient dialysis recoveries and a sensitive method of analysis. The blood microdialysis probe is easy to construct, and its surgical placement has a high success rate. Placement of the probe in the vena cava may be an advantage over placement in the jugular vein because of favorable flow characteristics for recovery and in this location the probe may not interfere with the blood supply to important organs. This study shows that the use of microdialysis blood sampling makes long-term cross-over experiments in one animal possible, which will not only decrease the number of animals used, but will also provide more detailed and accurate concentration-time information for pharmacokinetic studies.

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