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

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Received May 6, 1997; accepted July 1, 1997

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 T1/2, C1, 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 (Polymeric 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 (Filtrak 20, AN69, acrylonitrile/sodium methallyl sulfonate copolymer, I.D. 220 μm, O.D. 310 μm, Hospal, Cobol 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...
An intramuscular dose of 60,000 units procaine penicillin G was given following surgery. The vascular cannulae 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 online 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