Pharmacokinetic Monitoring in Subcutaneous Tissue Using *in Vivo* Capillary Ultrafiltration Probes

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Capillary ultrafiltration probes were utilized for *in vivo* sampling of therapeutic drugs in awake rats. Capillary ultrafiltration probes implanted into subcutaneous tissue were able to follow the disposition of acetaminophen and theophylline. Ultrafiltration probes provided samples at a rate of 2–3 μL/min. Ultrafiltrates were analyzed by liquid chromatography with either UV or electrochemical detection. Simultaneous ultrafiltration and microdialysis probes and multiple ultrafiltration probes were used in individual animals to validate the technique. The pharmacokinetics of two well-established drugs, acetaminophen and theophylline, were monitored in awake, freely moving rats to demonstrate the viability of the technique. The half-life for acetaminophen was determined to be 20.9 ± 1.0 min (n = 6) for a 2 mg/kg dosing. The half-life of elimination for theophylline was determined to be 3.0 ± 0.1 hr (n = 4) for a 4 mg/kg dose. The capillary ultrafiltration probes exhibited a constant flow rate of 2.4 ± 0.1 μL/min and removed 50 nL/min/mm of fluid from the extracellular space. Capillary ultrafiltration sampling is shown to be an excellent tool for *in vivo* monitoring of drug disposition and a suitable method for determining pharmacokinetic parameters in awake animals.

KEY WORDS: capillary ultrafiltration; *in vivo* sampling; pharmacokinetics; acetaminophen; theophylline; subcutaneous tissue.

INTRODUCTION

The monitoring of drug dynamics *in vivo* has become more practical since the development of microdialysis (1–5). Microdialysis has been shown to continuously monitor pharmacokinetics in freely moving awake rats (6,7). More recently, capillary ultrafiltration has shown potential for pharmacokinetic measurements (8,9). Capillary ultrafiltration probes are well suited for *in vivo* monitoring of drug disposition. Capillary ultrafiltration probes are implanted into the subcutaneous tissue, where water-soluble low molecular weight substances are collected from the extracellular space. The membrane prohibits the passage of protein and enzymes, making it possible to analyze the sample directly by liquid chromatography or another suitable analytical method. The probes allow for continuous sampling in awake, freely moving animals for several days after a single implantation.

Capillary ultrafiltration probes utilize a hydrophilic membrane fiber that allows for a negative pressure gradient to be applied across it by a peristaltic pump or other vacuum system. Intercellular fluid is pulled across the membrane and is collected. The probes are constructed of flexible materials that can be implanted into the subcutaneous tissue of any animal for extended periods of time. The ultrafiltration probes used here remove fluid from the extracellular space at an average rate of 50 nL/min/mm for at least 2 days after implantation. In the present study, a flow rate of 2.4 ± 0.1 μL/min was obtained. In contrast to the microdialysis technique, the percentage recovery or extraction fraction is not flow rate dependent and is typically greater than 95% (9). Comparison of two capillary ultrafiltration probes, as well as one ultrafiltration probe and one microdialysis probe, was carried out in a single animal to validate the technique.

Pharmacokinetic measurements of acetaminophen and theophylline were conducted using capillary ultrafiltration probes implanted into the subcutaneous tissue of rats. Ultrafiltrates were analyzed by liquid chromatography. Electrochemical detection was used for determination of acetaminophen, whereas UV detection was used for theophylline quantitation. Comparison of pharmacokinetic parameters obtained using microdialysis intravenously (6,7) corresponded well with the results obtained from subcutaneous sampling.

MATERIALS AND METHODS

Subjects and Surgery. Hooded Long Evans rats, 200–250 g (Harlan-Sprague Dawley, Indianapolis, IN), were used. The animals were housed in a temperature-controlled room under a 12-hr light/dark cycle. Food and water were provided *ad libitum*. Subjects were anesthetized with a 10:1 (100 mg/mL) mixture of ketamine–xylazine (1 mL/kg). A 5-mm incision was made in the back between the shoulders. A second incision was made in the back of the animal 5–7 cm posterior to the shoulders. A thin-walled 13-G needle was inserted through the two incisions. Either a UF-3-12 or a UF-3-16 capillary ultrafiltration probe, with three loops of polyacrylonitrile membrane 12 or 16 cm long and dimensions of 310 μm in o.d. and 220 μm in i.d. (Bioanalytical Systems, BAS, West Lafayette, IN), was inserted into the needle. In the dialysis experiments, a prototype loop microdialysis probe DL-6-PAN made with a 6-cm-long polyacrylonitrile membrane was used (10). The needle was then carefully removed, leaving the probe in the subcutaneous tissue. The probe was sutured to the skin to secure it. The two incisions were then sutured closed. The animal was then transferred to an awake animal sampling system and connected to the swivel. The animals were allowed to recover from surgery for 4 to 12 hr before experiments were conducted.

Awake Animal System. The awake animal system used is illustrated in Fig. 1. The animal is attached to a counterbalanced arm through a wire tether. The arm suspends either a single- or a dual-channel swivel (the dual-channel swivel was used only during microdialysis experiments). The combination of the swivel and swinging arm provides the animal with complete mobility about the cage. The capillary ultrafiltration probe is connected to the swivel with 110-μm-i.d. Teflon tubing (BAS). The swivel is likewise connected to a Minipulse 2 (Gilson, France) peristaltic pump. The peristaltic pump is equipped with 0.007-in. tubing with a total internal volume of 6.2 μL. The peristaltic pump was always set at

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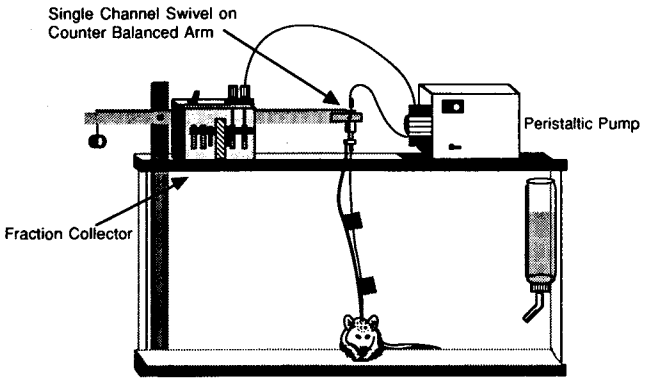

Fig. 1. Schematic of the awake animal system for capillary ultrafiltration sampling experiments. The system contains a peristaltic pump, a single-channel swivel on a counterbalanced arm, and an automated fraction collector.

the lowest setting, in our case 000, while still moving. Samples were collected using a CMA 140 fraction collector (BAS/CMA, West Lafayette, IN) and were analyzed immediately by liquid chromatography. Microdialysis was conducted with a CMA 100 microinjection pump (BAS/CMA).

Chromatography. Liquid chromatography was carried out with a BAS 200A chromatograph using a Biophase II C_{18}, 3-μm, 100 × 3.2-mm column at 35°C and a 1-mL/min flow rate. A 5-μL injection loop was used. For acetaminophen determinations, the mobile phase consisted of 75 m*M* monochloroacetic acid (pH 3.1), 0.67 m*M* EDTA, and 2.5% acetonitrile. Electrochemical detection for acetaminophen was accomplished using a 3-mm glassy carbon electrode maintained at a potential of +750 mV vs Ag/AgCl. For the separation of theophylline, a mobile phase of 100 m*M* phosphate buffer (pH 6.1) with 5% acetonitrile was used. Theophylline quantitation was accomplished using UV detection at 274 nm.

Materials. Acetaminophen and theophylline standards were purchased from Sigma (St. Louis, MO) and used as received. All buffers were made from analytical-grade materials purchased from Aldrich Chemical (Milwaukee, WI). HPLC-grade acetonitrile (Baxter, McGaw Park, IL) was used. All solutions were made with double-distilled deionized water and filtered through a 0.22-μm nylon filter.

In Vivo *Pharmacokinetic Experiments.* Ultrafiltrate samples were collected for at least 1 hr prior to all experiments. For acetaminophen, a 2 mg/kg dose in 0.5 mL of Ringer's was administered intraperitoneally. Theophylline was administered in a 4 mg/kg dose in 1 mL of Ringer's intraperitoneally. Samples were collected at timed intervals ranging from every 5 min for acetaminophen to up to 1 hr for theophylline. Microdialysis sampling was conducted by perfusing the probe at 3 μL/min with Ringer's solution. Animals were allowed food and water during the experiments.

RESULTS

In Vivo *Ultrafiltration Sampling.* Capillary ultrafiltration probes require no *in vivo* calibration due to the active process used in sample collection. *In vitro* calibration can determine the limiting interaction of a substance with the membrane and accurately calibrate the recovery (extraction fraction) (9). Theophylline was determined *in vitro* to have a 100.2 ± 0.8% (mean ± SE) recovery, whereas acetaminophen was determined to have a 97.6 ± 0.8% recovery.

Chromatographic Analysis. Due to the small sample volume, typically less than 20 μL, evaporation can become a significant problem (11). Thus, immediate analysis is desirable to limit errors in analyte determinations. Figure 2 illustrates typical chromatograms for ultrafiltration before injection of acetaminophen (A) and 20 min after injection (B). The separation of theophylline in ultrafiltrates is illustrated in Fig. 2 (C) before dosing and (D) 30 min after injection. Both methods elute substances of interest in less than 5 min.

Pharmacokinetic Parameters. The half-life of elimination was determined by plotting the log of the concentration against time (12,13). Linear regression analysis provided the constant β. The first-order elimination half-life ($t_{1/2}$) was determined by the equation 0.693/β. The initial concentration, $C(0)$, after a single dose was determined from the *y* intercept of the semilog plot. The area under the curve (AUC) values were determined using the trapezoidal method with extrapolation based on the terminal half-life beyond the last data point. The total-body clearance (CL) was calculated as the ratio of dose/AUC and the volume of distribution (V_d) was calculated as the ratio of CL/β.

Pharmacokinetics of Acetaminophen. Acetaminophen rapidly distributes throughout the body. For a 2 mg/kg dose, the maximum tissue concentration (ca. 1 μg/mL) is reached within 20 min. The concentration reaches undetectable levels within 200 min. Acetaminophen follows an open one-compartment model with first-order elimination and first-order absorption (6,13). The disposition of acetaminophen can be described by the following equation:

$$C_t = A_1 e^{-\beta t} - A_2 e - k_a t$$

where β is the elimination rate constant and k_a is the absorption rate constant. The pharmacokinetic parameters determined for acetaminophen using capillary ultrafiltration are listed in Table I.

Pharmacokinetics of Theophylline. Theophylline pharmacokinetics follows an open two-compartment model with first-order absorption (14). Theophylline distributes less rapidly than acetaminophen and reaches a maximum tissue concentration in about 30 min, with a peak concentration of 0.55 μg/mL (4 mg/kg dose). The half-life of elimination is quite long compared to that of acetaminophen, and detectable changes in concentration still occur after 14 hr. The disposition of theophylline can be described by the following equation:

$$C_t = A_1 e^{-\alpha t} + A_2 e^{-\beta t} - (A_1 + A_2) e - k_a t$$

where α is the fast distribution constant, β is the elimination constant, and k_a is the absorption constant. The absorption and fast distribution process are both occurring simultaneously. Therefore, it is difficult to evaluate the rate at which distribution occurs (12). Thus, only the slow disposition (elimination) half-life was determined for the β term. The disposition of theophylline in three separate animals is illustrated in Fig. 3. The pharmacokinetic parameters ob-

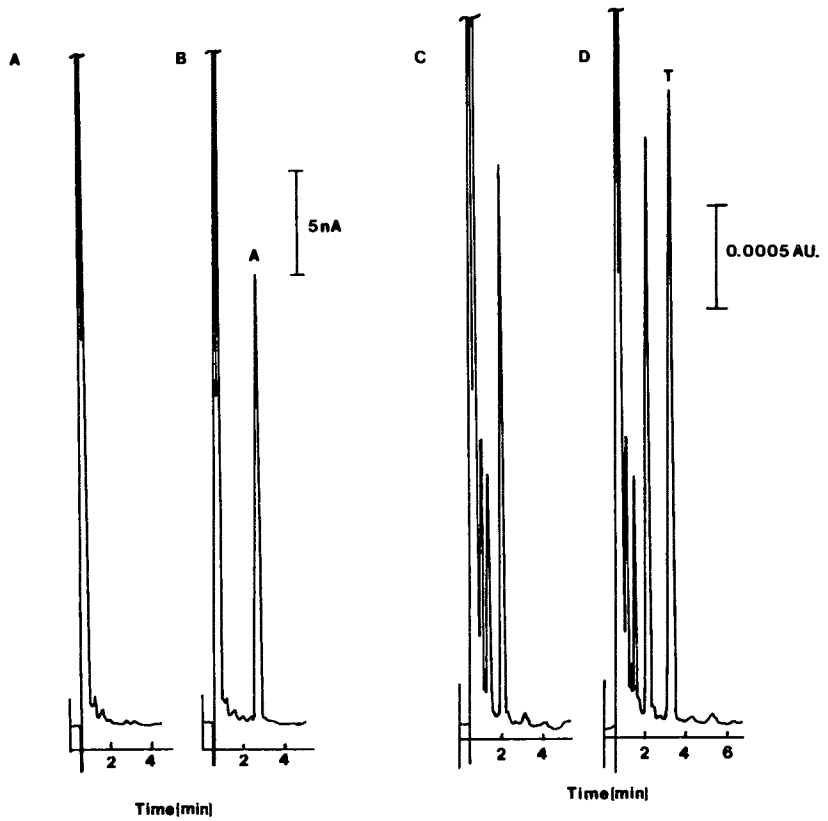

Fig. 2. Typical chromatograms obtained for ultrafiltrates of rat subcutaneous tissue. (A) An ultrafiltrate before injection of acetaminophen and (B) the acetaminophen peak after 30 min. (C) An ultrafiltrate before injection of theophylline and (D) the theophylline peak after 20 min.

tained for theophylline using capillary ultrafiltration are listed in Table II.

DISCUSSION

In order to be able to monitor drug disposition *in vivo*, the method needs to provide accurate time and concentration information. The capillary ultrafiltration probes used in this study allowed for quantitative collection of the drugs being monitored without sampling blood. The concentration in the sample is representative of the extracellular concentration. The capillary ultrafiltration probes utilized in this study provided steady flow rates. Five animals were implanted with UF-3-16 PAN probes and at least 10 samples were gravimetrically determined for each subject. A flow rate of 2.4 ± 0.1 μL/min (mean ± SE; n = 56) for the five probes was obtained from subcutaneous tissue. Samples acquired *in vivo* reflect an average concentration during the period of collection.

Comparison of Pharmacokinetic Parameters Determined by Intravenous Microdialysis with Capillary Ultrafiltration. Microdialysis has been previously used to monitor intravenous (6,7) and subcutaneous tissue (3–5) pharmaco-

Table I. Pharmacokinetic Parameters for Acetaminophen Dosed ip at 2 mg/kg[a]

$t_{1/2}$ elimination, min	20.9 ± 1.0
β, min^{-1}	0.0336 ± .002
AUC, μg min/mL	27.5 ± 3.4
V_d, L	0.59 ± .08
CL, mL/min	17.8 ± 3.5
$C(0)$, mg/L	0.85 ± 0.05

[a] n = 6 for all parameters. Mean ± SE.

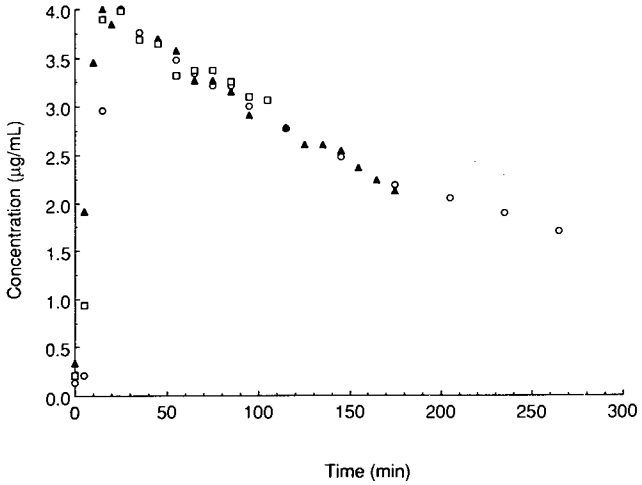

Fig. 3. The monitoring of theophylline disposition by capillary ultrafiltration in three animals with identical dosing (4 mg/kg).

Table II. Pharmacokinetic Parameters for Theophylline Dosed ip at 4 mg/kg[a]

$t_{1/2}$ elimination, hr	3.0 ± 0.1
β, hr^{-1}	$3.78 \times 10^{-3} \pm 7.0 \times 10^{-5}$
AUC, μg min/mL	1105.6 ± 74.9
V_d, L	0.24 ± 0.02
CL, mL/min	0.92 ± 0.06
$C(0)$, mg/L	4.50 ± 0.28

[a] $n = 4$ for all parameters. Mean ± SE.

kinetics. The results from this study, using capillary ultrafiltration, correspond very well to previously reported pharmacokinetic parameters for acetaminophen and theophylline. The elimination half-life for theophylline determined intravenously by microdialysis was 3.0 ± 0.23 hr ($n = 3$) (7), compared to 3.0 ± 0.1 ($n = 4$) for capillary ultrafiltration subcutaneously. The elimination half-life for acetaminophen determined intravenously by microdialysis (6) was 19.5 ± 1.33 min ($n = 3$), compared to 20.9 ± 1.0 min ($n = 6$) for capillary ultrafiltration. Again, these figures corresponded very well.

Comparison of Two Ultrafiltration Probes and Ultrafiltration with Microdialysis. Experiments were conducted in three animals with two identical ultrafiltration probes implanted contralaterally in each subject. In all cases, similar results were obtained from both probes. Figure 4 illustrates a representative disposition of acetaminophen monitored by two ultrafiltration probes in a single animal. The half-lives of elimination determined from these two probes, 23.1 and 21.2 min, were statistically indistinguishable (Student's t test, $\sigma = 0.5$).

Theophylline was monitored using both microdialysis and capillary ultrafiltration in subcutaneous tissue. It has been proposed that longer loop microdialysis probes have the potential of near 100% recoveries (10,15). In three animals, a loop microdialysis probe was implanted along with a capillary ultrafiltration probe. The microdialysis probe was perfused at 3 μL/min with Ringer's solution. Although at this flow rate the concentration in the dialysate was lower than

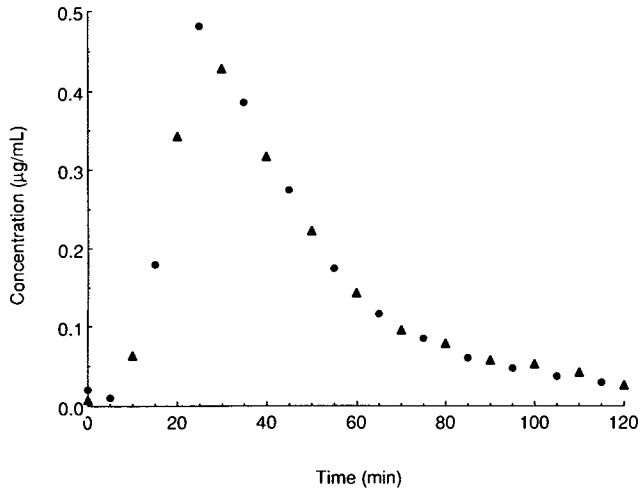

Fig. 4. Simultaneous monitoring of acetaminophen disposition in a single animal with two UF-3-16 PAN capillary ultrafiltration probes implanted in the subcutaneous tissue.

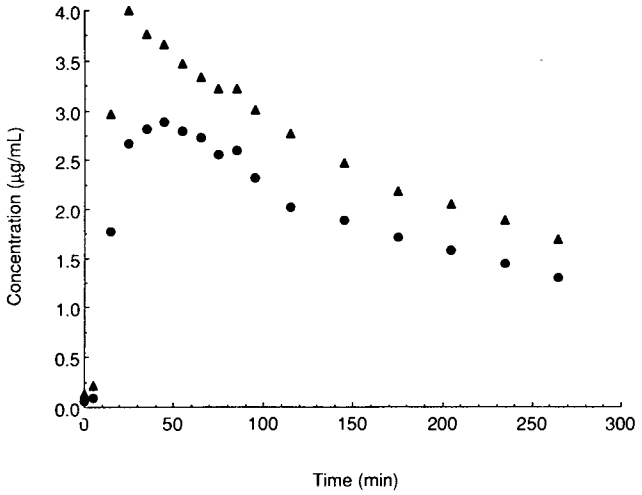

Fig. 5. Simultaneous monitoring of theophylline disposition in a single animal with a (▲) capillary ultrafiltration (UF-3-16) and (●) microdialysis probe (DL-6-PAN). The microdialysis probe was perfused at 3 μL/min with Ringer's solution.

that of the ultrafiltrate, both methods followed the disposition of theophylline similarly. A representative example is illustrated in Fig. 5. The half-lives of elimination for ultrafiltration and microdialysis were 3.2 and 3.3 hr, respectively, in this example.

CONCLUSION

Capillary ultrafiltration probes have been shown to be effective tools for monitoring pharmacokinetics in awake animals. The probes remove only a small amount of fluid from the extracellular space at a rate of 2–3 μL/min. Ultrafiltration is advantageous in that it provides a sample free of protein and quantitatively contains the small molecules of the extracellular fluid and requires no *in vivo* calibration, and an individual probe can be used for a number of days in a single animal. Two ultrafiltration probes in a single animal provide equivalent results. Capillary ultrafiltration provided pharmacokinetic data that corresponded well to data obtained using microdialysis. The effects of removing extracellular fluid at a rate of 50 nL/min/mm of membrane on the physiology are not known at this time. These subjects are topics of current research. The monitoring of glucose, ethanol, and antibiotics in both animals and fermentation reactors is currently being investigated. The long-term prospects for the technique will require study of a wide range of drug substances. These early results are promising, although it appears that this method will not be very useful for highly lipophillic substances.

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