Microdialysis Sampling for the Investigation of Dermal Drug Transport

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Microdialysis perfusion *in vivo* has the potential to be a powerful sampling technique in dermal and transdermal drug delivery studies. Characterization of a commercially available microdialysis probe *in vitro* considering relevant physiological parameters is a vital first step in the evaluation of microdialysis as a dermal sampling technique. In previous microdialysis studies, analyte concentration and neutrality have been implicated in altering microdialysis recovery. The recovery of a model compound 5-fluorouracil (5-FU) was investigated at several pH values and donor concentrations. The relative recovery of 5-FU by the microdialysis probe was affected by pH but not by donor concentration. To confirm further that the changing concentration and pH profile presented by the flux of 5-FU was not significantly altering microdialysis recovery, an experiment comparing direct and microdialysis sampling of a Franz diffusion cell receptor compartment was performed. Although the 5-FU concentration (0–686 ng/ml) and pH (7.40–7.24) changed substantially, the recovery of 5-FU was not adversely affected. To demonstrate the feasibility of dermal microdialysis, the flux of a commercial preparation of 5-fluorouracil was monitored utilizing a microdialysis probe implanted in excised rat skin *in vitro*. The results from the dermally implanted probe demonstrate the potential of the technique while establishing the limitations of the current microdialysis system.

KEY WORDS: microdialysis perfusion; 5-fluorouracil; dermal diffusion.

INTRODUCTION

The objective of transdermal delivery is to deliver medication to the systemic circulation with a minimum of dermal involvement, while the objective of dermal delivery is to deliver medication to the dermis with a minimum of systemic involvement. Transdermal administration of drugs has a number of advantages compared with other routes of administration such as minimization of first-pass effect, increased patient compliance, reduction of side effects or loss of therapeutic efficiency, and avoidance of bioavailability problems (1,2). Topical application of a drug allows localized delivery of a therapeutical agent directly to the site of action while reducing the number and extent of systemic effects.

Typically, dermal and transdermal delivery systems are evaluated using isolated skin preparations *in vitro*. Substantially differing diffusional and metabolic profiles have been shown in skin between experimentation *in vivo* and *in vitro* (3). Methods for the analysis of transdermal delivery *in vivo* involve radiolabeled compounds measured in plasma or urine (4). Neither methods using diffusion cells *in vitro* nor methods measuring appearance in the blood or urine can directly determine the flux of drug through the dermis. Clearly, these techniques cannot directly address the issues of dermal retention or metabolism.

Microdialysis is an *in vivo* sampling technique which causes minimal tissue damage or physiological alterations to the test subject. Advantages of microdialysis sampling are that, once a molecule crosses the dialysis membrane, no enzymatic degradation or protein binding can occur and that tissue can be directly sampled without significant tissue fluid loss (5). In general, sampling rates can be optimized with microdialysis to minimize thermal or hydrolytic degradation of the compounds of interest.

Whereas the majority of microdialysis studies has focused on neurotransmitters and other compounds in the central nervous system, several investigators have used microdialysis successfully to monitor systemic fluids and tissues in man and the rat (6–9). In addition to determining the systemic levels of drugs, it was possible to observe the physiologic response of endogenous compounds following stimulation by drugs and various physiologic conditions (6–9). Recently, microdialysis has been used for pharmacokinetic investigations using both intravenous and tissue sampling (10–13).

The dermal microdialysis technique may permit a significant reduction in the number of animals required to perform dermal and transdermal drug delivery research. With microdialysis, a single animal can be used for the continuous real-time study of drug flux through the skin, compared with the numerous animals currently required for each time point. A reduction in experimental error can also be realized because each animal acts as its own control, thereby reducing the interanimal variation and the number of animals required to achieve appropriate statistical significance.

This report demonstrates the ability of microdialysis to monitor directly the dermal flux of a topically applied compound. 5-Fluorouracil (5-FU) was selected as a model compound to evaluate intradermal microdialysis because of the established knowledge about the metabolism, kinetics, dermal flux, and physiological effects of 5-FU (14–17). This knowledge of topically and systemically administered 5-FU provides an excellent comparative basis for the assessment of the microdialysis technique.

EXPERIMENTAL

Materials and Reagents

5-Fluorouracil and calcium chloride were obtained from Sigma Chemical Company (St. Louis, MO). Potassium chloride was obtained from MCB Manufacturing Chemists (Cincinnati, OH). All other chemicals were obtained from Fisher Scientific (Fair Lawn, NJ). All chemicals were used as received. Gentamicin sulfate was stored refrigerated. All water

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was processed through a Milli-Q water system (Waters Corp., Bedford, MA). Efudex 5% Cream was a gift from Hoffmann-LaRoche, Nutley, NJ.

Analysis of 5-FU

Analysis of 5-FU was based on the previous method of Christophidis *et al.* (18). The chromatograph consisted of a Model LC-6A pump, a Model C-R5A integrator, a Model SPD-2 UV-VIS spectrophotometric detector, and a Model SCL-6A system controller (Shimadzu Corp., Kyoto, Japan) and a Model CMA 160 Injector (Bioanalytical Systems Inc., West Layfayette, IN). A Panasonic Model TR-120 MDPA (Matsushita Electronic Industry Co. Ltd., Osaka, Japan) video monitor and a model FDD-1A dual floppy drive (Shimadzu Corp., Kyoto, Japan) were used for data analysis and storage. A column switching system consisting of the above chromatographic system with the addition of a second Model LC-6A pump and a FCV-2AH 6-port switching valve (Shimadzu Corp.) was used for experiments involving excised skin. Separation was achieved using two ODS Hypersil columns (each 5 μm, 4.6 × 15 cm, Keystone Scientific, State College, PA). A 6.6-μl injection loop attached to a CMA/160 on-line injector (Bioanalytical Systems Inc.) was used for on-line sample injection. A 500-μl syringe was used for manual injections (Kloen, Brea, CA) using the CMA 160 injector. For all experiments, the column temperature was kept at 35°C using a Model 7960 column heater (Jones Chromatography, Littleton, CO). Detection of 5-FU was performed at 260 nm for all analyses.

Samples were injected directly into the appropriate chromatographic system without any prior preparation. Samples collected manually from the Franz diffusion cells were stored immediately in a −20°C freezer until analysis. In the isocratic mode used for nonbiological samples, a single ODS column was eluted with KH_2PO_4/H_3PO_4 (50 m*M*, pH 3.5) at a flow rate of 1.5 ml/min. Due to chromatographic interferences, a column-switching scheme (ODS-ODS) was utilized to achieve satisfactory separation for samples derived from excised skin (Fig. 1). The first ODS column was eluted with KH_2PO_4 (25 m*M*, pH 4.5), while the second ODS column was eluted with KH_2PO_4/H_3PO_4 (50 m*M*, pH 3.5), both at a flow rate of 1.5 mL/min. The switch window between the two columns was 8.4 sec. Microdialysis samples were collected in the injection loop and injected directly into the chromatography (Fig. 2). Typical calibration data satisfied the following linear equations for nonbiological sample analysis, 25 ng/ml to 100 μg/ml, $y = 12777x + 167$ $r^2 = 1.00$, and for biological sample analysis, 50 ng/ml to 10 μg/ml, $y = 2253x - 11.6$ $r^2 = 0.998$, 5 to 100 μg/ml, $y = 1815x + 2597$ $r^2 = 0.997$.

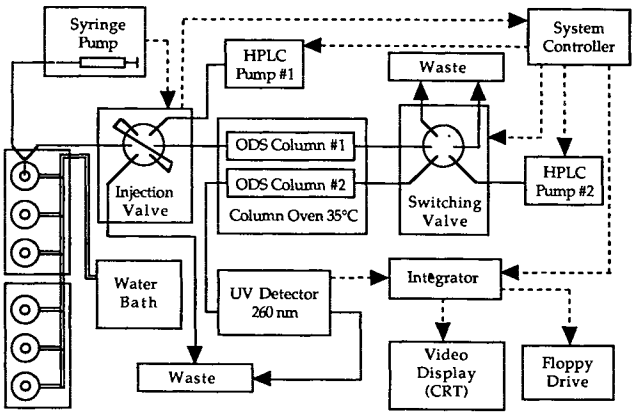

Fig. 1. Microdialysis, Franz diffusion, and chromatographic systems.

Microdialysis System

The microdialysis system used throughout this investigation consisted of a CMA/100 syringe pump, CMA/160 on-line injector, *in vitro* calibration stand, FEP tubing, flanged tubing adapters, and CMA/11 microdialysis probes (4-mm membrane length) (Bioanalytical Systems Inc.). Probes were connected to the injector and perfusion syringe with 20-cm lengths of FEP tubing containing a dead volume of 2.2 μl. A pair of 1-ml syringes (Eximer, Ito Corp., Fuji, Japan) was used with the CMA/100 pump to provide the perfusate for all microdialysis experiments. The CMA/160 on-line injector was programmed for a 5-sec injection.

Probe Preparation and Storage

Upon initial use, each microdialysis probe was perfused with and soaked in 80% ethanol. Prior to experimentation, a probe was perfused with at least 0.5 ml of water while soaking in the same solution, after which the probe was soaked in and perfused with at least 0.5 ml of the appropriate buffer solution. Whenever a perfusate syringe was mounted or changed, the dialysate flow was ramped up to 15 μl/min or more for the equivalent of at least three injector loop volumes. After use, probes were rinsed and perfused with at least 0.5 ml of water before storage in water between experiments.

In Vitro Dermal Diffusion System

The *in vitro* dermal diffusion system consisted of six improved Franz diffusion cells, caps, and clamps mounted onto two three-unit stirring bases equipped with glass manifolds (Crown Glass, Somerville, NJ). Either a Model L-D2 Haake (Berlin, Germany), a Model 80T VWR (VWR Scientific, San Francisco, CA), or a Model RMT6 Lauda (Brinkman Inst., San Francisco, CA) circulating water bath was used to provide temperature control for the water-jacketed Franz diffusion cells.

Skin Preparation

Dorsal skin was excised from adult male Sprague Dawley rats weighing approximately 500 g. The animal was first shaved in the direction of the fur, then in the opposite direction using an Oster (Milwaukee, WI) Model A2-O1E animal clipper (blade size 40), taking care not to damage the skin. Upon excision, the skin was washed with Dulbecco modified phosphate-buffered saline (DMPBS) and any excess connective or adipose tissue removed (19). The skin was washed with DMPBS throughout the procedure and soaked in DMPBS when not undergoing preparation. The skin excision and preparation procedure was completed as expediently as possible to maintain skin viability and integrity. The skin

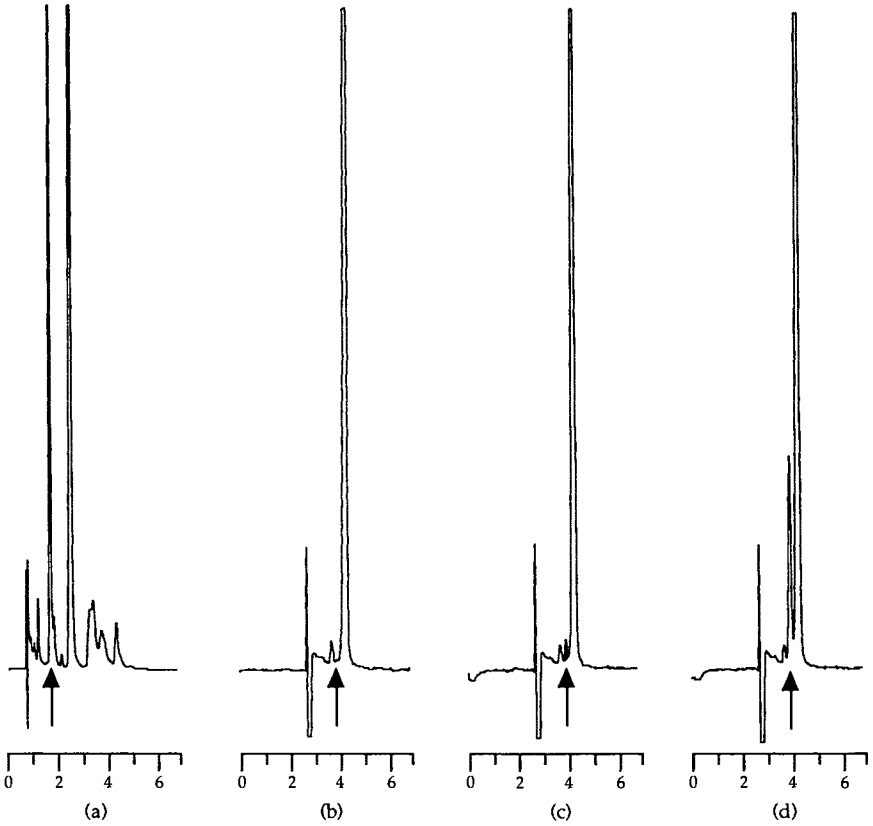

Fig. 2. Unswitched (a) and switched (b) chromatograms of blank Franz receptor matrix after 24 hr (c) and a chromatogram of 1 μg/ml 5-FU spiked into the 24-hr Franz diffusion-cell donor matrix (d). Arrows denote time of 5-FU elution.

was then cut to an appropriate size for mounting on the Franz diffusion cell.

Franz-Cell Preparation

Prior to use, Franz diffusion cells were rinsed with water and then filled with DMPBS. The buffer remained in the cell for approximately 20 min and was removed and replaced by new buffer solution. The new solution was then allowed to equilibrate thermally before skin samples were mounted.

Probe Implantation

Skin samples for probe implantation were prepared as previously described, except prior to mounting, a microdialysis probe was inserted into the skin. Probe insertion was accomplished by the use of a 21-gauge hypodermic needle, cut, and filed to measure 14 mm from tip to base, which served as a guide cannula. After inserting the guide cannula, the skin was visually inspected for any damage or punctures. The microdialysis probe was then inserted into the guide cannula and the guide cannula slid back along the probe shaft to the base of the probe exposing the dialysis membrane. The skin specimen was then placed on the Franz diffusion cell, centering the portion of the skin with the dialysis membrane over the receptor opening. The glass Franz diffusion cell cap was then carefully clamped onto the flanged base of the cell, effectively sandwiching the skin–cannula system.

Diffusion-Cell Experiments

Skin samples were mounted on top of the flanged portion of the Franz diffusion cell, onto which the top glass cap was clamped. The skin was allowed to equilibrate for approximately 5 min before a blank sample was taken, after which drug was applied. An excess of Efudex cream was applied to an approximate thickness of 5 mm to appropriate skin samples. The tops of the Franz cells were loosely covered with plastic caps to prevent evaporation of solvent from the Efudex cream. All skin diffusion experiments were performed at an equilibrated Franz-cell receptor temperature of 32°C. Approximately 100 μl of Franz diffusion-cell receptor phase was sampled using a 500-μl Hamilton gas-tight syringe (Hamilton Syringe Co., Reno, NV). Samples of the receptor phase were obtained initially and then every 30 min for the first 6 hr, after which samples were taken at 8, 12, 18, and 24 hr. Receptor-phase samples were placed in plastic vials. Each sample was immediately replaced with an equal volume of buffer.

Franz Receptor Concentration Monitoring

Skin samples were prepared as described previously, except prior to mounting, a microdialysis probe was inserted into the receptor portion of a modified, improved Franz diffusion cell (Fig. 3). The dialysis membrane portion of the probe was visually centered in the Franz-cell receptor. The probe was mounted in the Franz cell using dental cement and

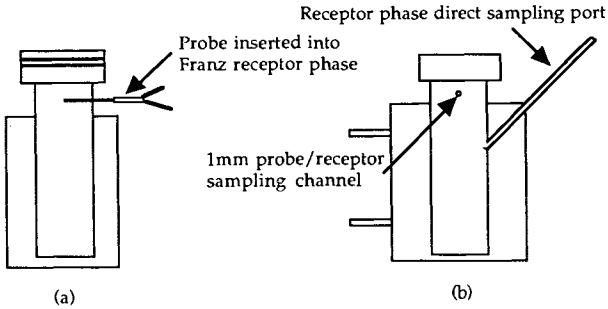

Fig. 3. Modified improved Franz diffusion cells. (a) View of modified Franz diffusion cell displaying probe inserted into receptor; (b) sideview of modified Franz diffusion cell displaying the position of the 1-mm microdialysis probe sampling channel.

Superglue. A magnetic stir bar was placed in the receptor and rotated at 600 rpm throughout the experiment. The glass Franz diffusion-cell cap was then carefully clamped onto the flanged base of the cell.

RESULTS AND DISCUSSION

The initial phase of research was concerned with identifying factors which could affect the microdialysis recovery of 5-FU under both *in vitro* and *in vivo* conditions. The factors selected for investigation were donor concentration and pH. The ability of a microdialysis probe to monitor a changing 5-FU concentration–time profile accurately was then investigated, followed by the dermal implantation of a microdialysis probe.

Analyte Concentration

The effect of analyte concentration on recovery was studied by sampling four known concentrations of 5-FU dissolved in DMPBS. The relative recovery was determined for each analyte concentration. Relative recovery was independent of analyte concentration over the concentration and perfusion rate ranges studied (Fig. 4). The relative recovery can therefore be used to determine the actual sample concentration from the dialysate concentration, meaning that the relative recovery is, in effect, the response factor of the dialysis probe.

pH

The effect of pH on recovery of 5-FU was evaluated using a 50 m*M* phosphate buffer (μ = 0.15). 5-FU is a weak acid with pK_a values of 8.0 and 13 (20,21). Recovery of 5-FU decreased as the solution pH increased, consistent with the ionization profile of 5-FU (Fig. 5). The higher recovery of the neutral form of 5-FU is indicative of faster diffusion through the dialysis membrane. A similar selectivity of the dialysis membrane for neutral species has been observed previously (5,10,22).

Effect of Concentration and pH on Experimentation *in Vitro*

The amount of 5-FU in the Franz receptor increased with time as 5-FU diffused through the skin, changing the receptor pH from an initial value of 7.40 to 7.26 after 24 hr. Considering the effect of pH on probe recovery and the change in receptor pH, the probe concentration–time recovery profile may be altered as the pH decreases. The effect of changing pH was evaluated by mounting a probe in a Franz-cell receptor and comparing the resultant concentration–time profiles of directly sampled receptor with those of receptor microdialysate. After an initial lag phase, both the receptor and the dialysate exhibited increasing 5-FU concentrations (Fig. 6). The relative recovery at a flow of 0.66 μl/min in 740 ng/ml of 5-FU in skin receptor medium was found to be 53%. When the dialysate concentration was multiplied by the recovery factor (1/0.53), the corrected concentration–time profile determined by the dialysis probe was superimposable on the profile observed for the receptor.

Dermal Microdialysis

The next phase of research was to establish that a microdialysis probe implanted in the skin could monitor dermal drug flux. Following the outlined procedure, a microdialysis probe was implanted in the skin mounted on a Franz diffusion cell. Samples were collected for 15-min intervals for 24 hr and receptor-cell samples were collected every 30 min for the first 6 hr, then after 8, 12, 18, and 24 hr. Topical appli-

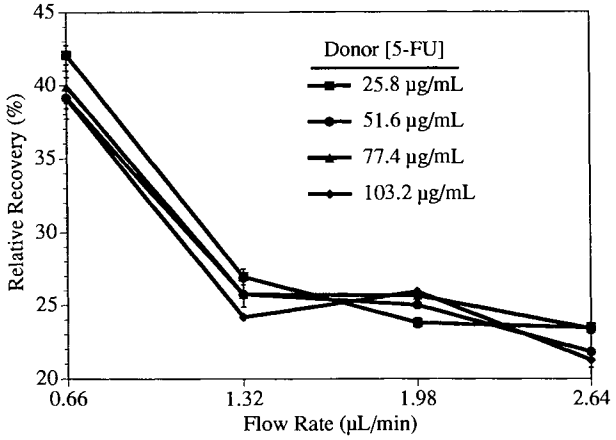

Fig. 4. Relative recovery of 5-FU at various donor concentrations (average of three replicates; error bars indicate standard deviation).

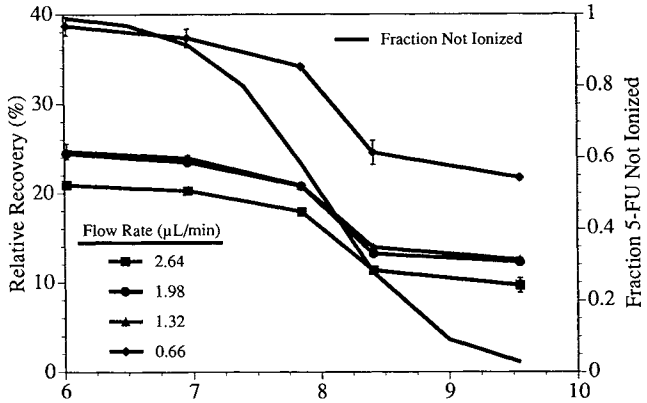

Fig. 5. Effect of pH on 5-FU recovery at various flow rates and the ionization of 5-FU at various pH's; species not ionized represented (average of three replicates; error bars indicate standard deviation).

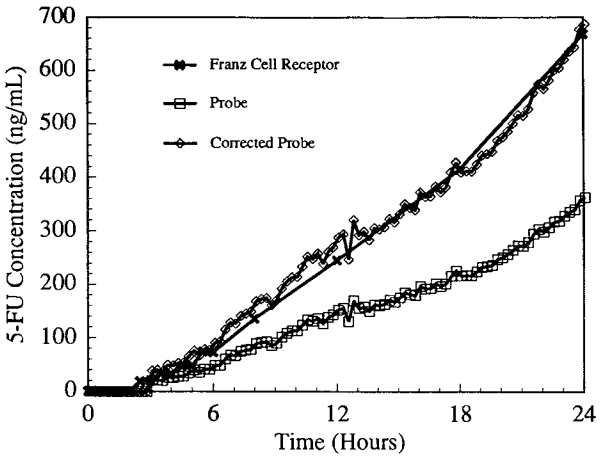

Fig. 6. *In vitro* receptor concentration–time profiles.

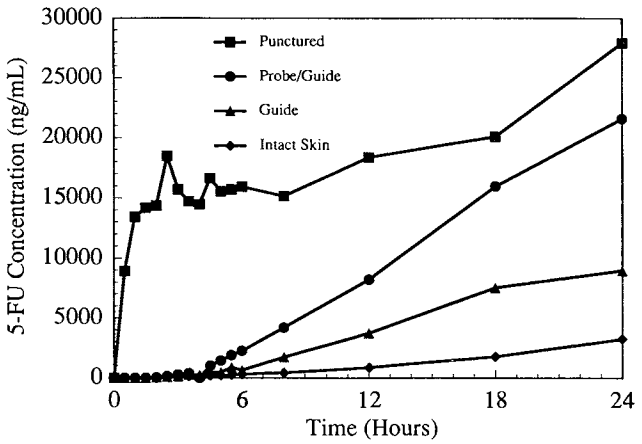

Fig. 8. Resulting receptor concentrations from simultaneous Franz diffusion-cell experiments.

cation of 5-FU (Efudex) resulted in an initial rapid increase in 5-FU concentration in the dermis for the first 6 hr, after which steady-state levels were observed until completion of the experiment (Fig. 7). The Franz-cell receptor phase showed an initial lag phase, followed by an increase in concentration over the 24-hr period.

Franz Diffusion-Cell Experiments

Franz diffusion-cell experiments were performed to determine the flux of 5-FU through the skin and to assess any damage to the skin caused by dialysis probe insertion (Fig. 8). Two controls with no dialysis probe were run to determine the dermal 5-FU flux through skin mounted in the Franz cell in the abscence of a probe. These two controls exhibited similar concentration–time profiles (intact skin). A second control experiment involved puncturing a hole down through the skin (puncture) with a 21-gauge needle to determine the flux profile through damaged skin. The cell containing the punctured skin showed an immediate and persistent level of 5-FU in the receptor phase. Experiments were then performed using excised skin mounted in a Franz diffusion cell with just a guide cannula (cannula) and with a probe inserted (cannula/probe). In both cases, increased 5-FU flux was observed relative to the intact skin controls but lower than the punctured skin control. These results indicate that implantation of the cannula increased the permeability of 5-FU through the skin, possibly by disrupting the dermal structure and providing a "pocket" through which enhanced flux of 5-FU may occur without actually puncturing the skin.

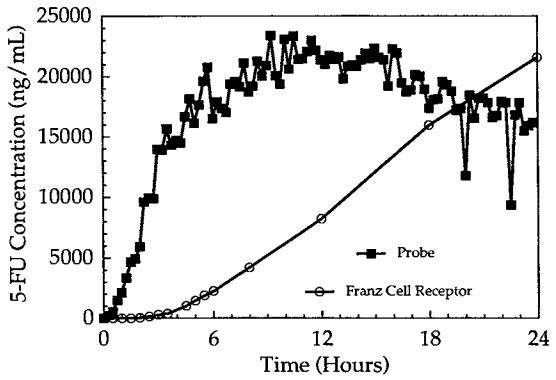

Fig. 7. Concentration-versus-time profile of a dermally implanted probe.

CONCLUSIONS

The ability to monitor a changing drug concentration–time profile in skin by microdialysis has been demonstrated. A system utilizing a modified Franz diffusion cell for the evaluation of microdialysis probe performance *in vitro* has also been developed. To ensure constant recovery through the probe, both the sample pH, or more appropriately, the ionization of 5-FU, and the perfusion flow rate must be carefully controlled. As previously reported, analyte concentration had no effect on recovery (10,20). Neither factor, concentration nor pH, had any noticeable effect on the performance of the probe in the Franz-cell receptor study *in vitro*. Microdialysis sampling of the Franz-cell receptor, when corrected for probe recovery, resulted in a concentration–time profile identical to that of the direct sampling of the Franz-cell receptor.

Although the implantation of the probe affected the flux of 5-FU through the skin, the implanted probe exhibited a reasonable concentration–time profile for the dermal layer with respect to the concentration–time profile of the Franz-cell receptor phase. The current results suggest that the size of the guide cannula may be excessive, apparently disrupting the dermis and affecting the flux of drug through the surrounding tissue. Any adverse effects due to the size of the guide cannula can be minimized by a reduction in its dimensions. The successful dermal implantation of a microdialysis probe provides evidence of the impending ability of dermally implanted microdialysis probes to monitor directly the dermal flux of drugs. Work in these laboratories is currently under way to develop microdialysis probes that will not alter the dermal flux of drugs through the skin. When perfected, the dermal microdialysis technique will provide a method for the evaluation *in vivo* of dermal and transdermal drug delivery.

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