Monitoring Transdermal Delivery of Nicotine Using In Vivo Microdialysis Sampling

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A linear microdialysis probe was implanted into the dermis of rats. A small piece of nicotine patch was affixed on the skin to achieve transdermal delivery of nicotine. Microdialysis samples were collected continuously for up to 24 hours. Dialysate samples were analyzed on-line by LC-UV to determine the concentrations of nicotine in the dialysate. The peak levels of nicotine were detected between 2 and 5 hours after patch application. The results reveal a temporal profile of nicotine transdermal delivery and demonstrate the usefulness of the linear probe for transdermal drug delivery studies.

Microdialysis sampling in vivo is gaining acceptance in pharmacological studies (1,2). Numerous studies have demonstrated the capability of microdialysis sampling in characterizing the behavior of exogenous compounds in tissues such as blood (3-6), muscle (7,8), solid tumor (9), adipose (7,10), liver (11-13), and dermis (14-17). Of all the commercially available probe designs, the BAS linear tissue probe (***F1***) is especially suitable for use in tissues other than the brain. The usefulness and reliability of the probe has been demonstrated in muscle tissue using acetaminophen as the test compound (18).

The pharmaceutical industry has recently placed increasing emphasis on dermal delivery of drug entities, especially timed-release patches. These new delivery formats, including dermal patches, have increased the sales of some established and generic drugs. Such transdermal administration of drugs has a number of advantages compared with other routes of administration. These include increased patient compliance, minimization of first-pass effect, reduction of side effects or loss of therapeutic efficiency, and avoidance of bioavailability problems. Topical application of a drug allows localized delivery of a therapeutic agent directly to or near the site of action while reducing the number and extent of systemic effects.

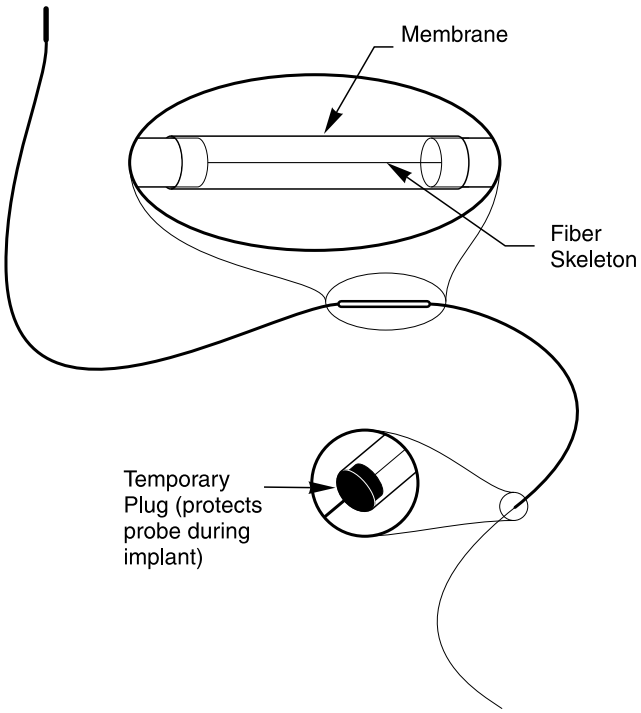
Traditionally, pharmacokinetics of topically administered drugs are obtained in vivo by measuring and calculating drug concentrations from serial blood samples. Among the disadvantages of this approach are poor temporal resolution because of the limitations of blood sampling intervals and the extensive sample preparation required prior to analysis.

Microdialysis sampling offers several features that overcome these limitations. The microdialysis process does not change the net fluid balance, so continuous sampling can be performed. Temporal resolution of the experiment is then determined by the volume of sample needed for analysis and the detection limit of the analytical method, or by the analysis time if the sampling is carried out on-line. Microdialysis is essentially a size exclusion process, so the dialysate is a protein-free aqueous sample amenable to direct injection for analysis by LC. The microdialysis probe implanted in the dermis samples from the extracellular fluid and thus reflects the local profile of the drug. Recently, this technique has been used in dermal pharmacokinetic studies in humans and animals (14-17).

Various linear microdialysis probe designs have been used for studies in different tissues (9,14). The excellent flexibility of the BAS linear tissue probe, along with its fiber skeleton for strength and ease of implantation, makes it well-suited to demonstrate the potential of microdialysis sampling as a tool for the study of transdermally delivered compounds. In this report, we chose to monitor the flux of nicotine from a dermal patch intended to assist in the cessation of smoking.

F1

The BAS Linear Tissue Probe.



Methods And Materials

Chemicals

Nicotine was purchased from Sigma (St. Louis). Nicotine patches (Nicotrol®, McNeil Consumer Products Co., PA) were purchased from Watkins Health Center (Lawrence, KS). HPLC grade acetonitrile was obtained from Fisher Scientific (Fairlawn, NJ). All standards and solutions were prepared using purified water obtained from a Barnstead Nanopure System. All other chemicals were reagent grade or better and used as received.

Liquid Chromatography

The LC system consisted of a BAS 200 with a UV-116 detector operated at 260 nm (BAS, West Lafayette, IN, USA). Separation was achieved on a 3 µm phenyl column (2.1 mm x 100 mm) (BAS). The mobile phase was 0.05 M sodium phosphate buffer, pH 3.3, containing 20% acetonitrile by volume and 30 mM SDS, at a flow rate of 0.8 mL/min. The limit of detection for nicotine was 1 µM.

Microdialysis System

The probe was perfused with Ringer's solution (155 mM NaCl, 5.5 mM KCl, 2.3 mM $CaCl_2$) at 1 µL/min using a syringe pump. Dialysate was collected directly into the loop (5.5 µL) of an automated injection valve connected to the BAS 200 system.

Surgical Procedure

Male Sprague-Dawley rats weighing 400-450 g were anesthetized intramuscularly using ketamine and xylazine (80 mg/kg and 10 mg/kg, respectively). An area on the back of the rat was closely shaved and cleaned with surgical scrub and rubbing alcohol. Using aseptic techniques, a linear probe (custom membrane window length of 20 mm, BAS) was implanted in the dermal tissue of the back by inserting a 22-gauge needle through the dermis and inserting the fiber extension of the probe through the needle. The needle was then withdrawn and the probe was pulled through the tissue, placing the dialysis membrane fully inside the dermis. The probe inlet and outlet tubes were tunneled under the skin and externalized at the center of the back of the neck. The fiber extension with its glue plug was cut away and the connector was attached to the probe tubing. Following surgical procedures, the rat was maintained in a BAS BeeKeeper awake animal system, which allowed movement without tangling fluid lines. The rat had free access to food and water throughout the experiment.

In Vivo Delivery of Nicotine Via the Probe

In vivo delivery was determined by perfusing the probe with a solution of 40 µM nicotine in Ringer's solution. The delivery experiment began 3 to 4 hours after surgery (day 0) and was repeated daily. Delivery, *D*, was calculated according to the following equation:

$$D = \frac{C_i - C_d}{C_i} = 1 - (\frac{C_d}{C_i})$$

where C_i is the initial concentration of nicotine entering the probe, and C_d is the nicotine concentration in the dialysate exiting the probe.

Transdermal Nicotine Dose Via the Patch

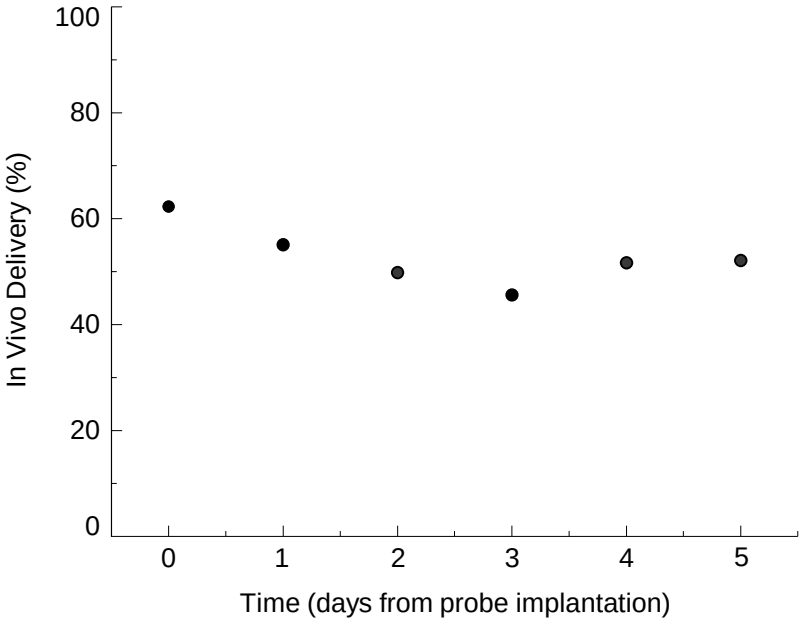
After 3 hours of baseline sampling, during which no interfering peaks appeared in the dialysis samples, a section of nicotine patch (containing ca. 2 mg) was affixed to the dermis above the implanted probe. We estimate that the average dose was about 5 mg/kg/24 hrs. Dialysate samples were continuously collected and automatically injected into the LC system at 8 min intervals for the first 5 hours and at 30 min intervals for the rest of the experiment, up to 24 hours. The concentration of nicotine in the dialysate was calculated from a standard curve.

Results And Discussion

The results of daily in vivo delivery of nicotine via the probe are shown in ***F2***. Except for the initial delivery on day 0, which began about 3 hours after probe implantation, the values are fairly uniform. Changes in circulation as an initial response to probe implantation

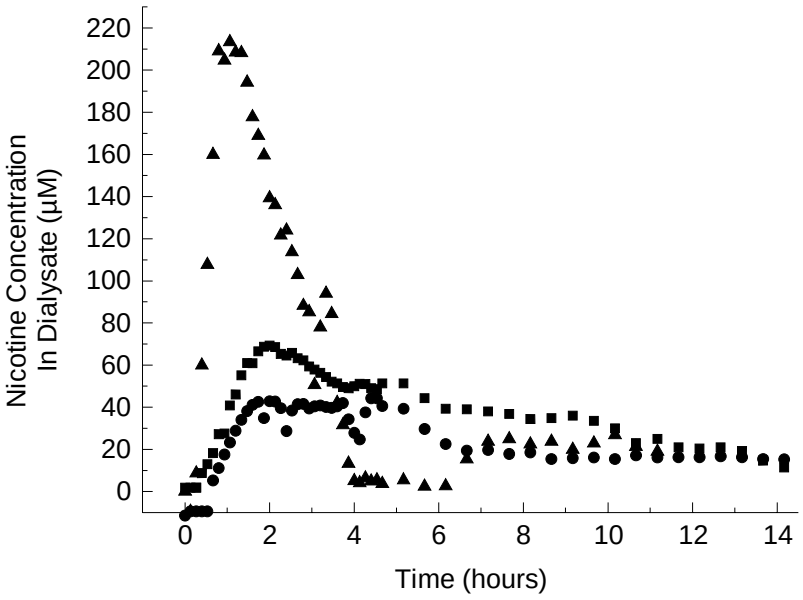
F2

In vivo delivery of nicotine via a microdialysis probe implanted in the dermis of a rat. Points are average relative delivery based on at least four consistent sequential injections.



F3

Concentration-time profile of nicotine as monitored by a microdialysis probe in the dermis of rats (▲ rat 1, ■ rat 2, ● rat 3). A patch containing ca. 2 mg nicotine (average dose 5 mg/kg/24hrs) was applied above the probe window.



might account for the higher initial delivery value. Anderson et al., using laser Doppler perfusion imaging, studied local changes in circulation around the site immediately following microdialysis probe implantation in skin (15). After implanting a probe in the forearm skin of human subjects, they found a rapid increase in local circulation around the probe that persisted for about one hour. Although the day 0 delivery was carried out about 3 hours after probe implantation, altered local circulation might be involved since the anesthesia probably modulates time frame for circulatory changes. Ault et al. (14) reported a significant decrease in probe delivery roughly correlating to acute inflammatory response in the skin between 24 and 48 hours after implantation. After the sharp decrease, Ault et al. reported stable delivery values. While the data presented in ***F2*** shows gradual decreases in delivery from days 1 through 3, the deliveries on days 4 and 5 increase to about the level observed on day 1.

F3 shows the temporal profile of nicotine concentration in the extracellular fluid of the skin for three different rats. Nicotine was first detected at 40-60 min after placing the nicotine patch on the skin. The peak levels of nicotine were seen within 2-5 hours after patch application. The maximum levels of nicotine in the dialysate showed large interindividual differences, ranging from 60 µM to 200 µM. Hegemann et al. reported large interindividual differences when using microdialysis sampling to monitor nicotine from patches applied to humans (17). Wide individual variations were also reported by Ault et al. for 5-fluorouracil flux in rat dermis using in vivo microdialysis (14). When implanting a probe into the skin, it is difficult to obtain placement at exactly the same depth in each case. Thus, the microcompartments surrounding the probes may vary among the subjects. These differences could account for the wide interindividual variation in maximum concentration. Alcohol has been shown to enhance skin penetration of compounds (19), so differences in quantity and length of exposure to alcohol during the surgical scrubbing process could also contribute to the individual variations.

As can be seen in ***F3***, the nicotine levels in the dermis of rats 2 and 3 (■ and ●) remain relatively stable (near the peak concentration) for about 2 hours before gradually declining. The pattern for rat 1 (▲) shows a plateau lasting only one hour followed by a very rapid decline in nicotine concentration in the dialysate. Between 4 and 7 hours after application of the patch, nicotine concentration in the dialysate was less than 10 µM and then increased again to around 20 µM, which is about the same level as seen in the other two rats between 7 and 14 hours. Inconsistent contact of the patch to the skin would account for this pattern and for the dip in concentration during the plateau for rat 3 (●) between 4 and 5 hours.

This research demonstrates the potential of microdialysis sampling in monitoring penetration of compounds through the skin. It also shows the reliability and durability of the BAS linear tissue probe implanted in dermis.

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