CAPSULES notes and applications from BASi

334

March 1998

In Vivo Microdialysis Sampling in Skin: Monitoring Nicotine from a Dermal Patch

Introduction

Purpose: The pharmaceutical industry has recently placed increasing emphasis on transdermal drug delivery systems. In vivo microdialysis sampling coupled on-line to liquid chromatography demonstrates the utility of the linear probe for monitoring transdermal flux of nicotine.

Existing Methods: Ex vivo monitoring of dermal drug delivery used excised skin mounted on a Franz cell. Topically applied drug passes through the skin to the receptor chamber where samples are removed for analysis. In vivo, pharmacokinetics of topically administered drugs are traditionally monitored by serial blood samples. Among the disadvantages of this method are poor temporal resolution because of limitations of blood sampling intervals and the extensive sample preparation required prior to analysis.

Microdialysis Sampling Method: The initial development of microdialysis sampling took place in the neurosciences and the success of the technique has led to its application for physiological and pharmacological studies in other tissues. Of all the commercially available probe designs, the BAS linear tissue probe ***[F1]*** is especially suitable for use in tissue other than the brain. By implanting the probe in the dermal layer, the flux of nicotine from a dermal patch can be monitored in an awake animal.

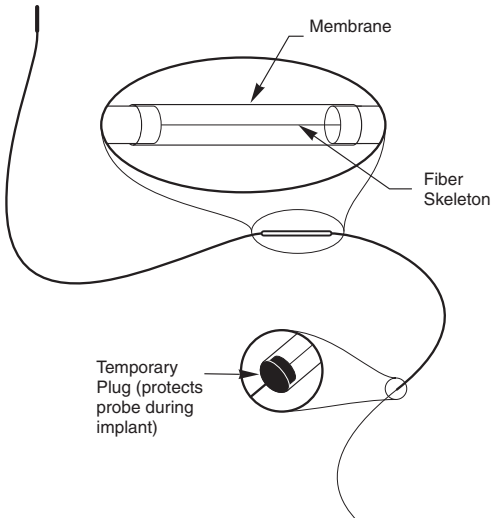

Figure 1. *The BAS Linear Tissue Probe.*

Experimental

Sample Analysis: The LC system consisted of a BAS 200 with an external UV-116 detector operated at 260 nm. Separation was achieved using a 100 x 2.3 mm, 7 mm phenyl column [MF-6254]. The mobile phase was 50 mM sodium phosphate buffer, pH 3.3, containing 20% acetonitrile by volume and 30 mM SDS. 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. Dialysate was collected directly into the 5.5 µL loop 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 thoroughly cleaned with surgical scrub and rubbing alcohol. Using aseptic technique, a BAS linear microdialysis probe (custom membrane length 20 mm) was implanted in the dermal tissue of the back by inserting a 22 gauge needle through the dermis. The fiber extension was threaded into the needle and the needle was withdrawn. The probe was drawn into place so that the membrane was fully embedded in the dermis. The probe inlet and outlet tubes were tunneled under the skin and externalized at the back of the neck. The fiber extension and glue plug were cut away and the connector attached as described in the probe user's guide. Following surgery, the rat was maintained in a BAS BeeKeeper awake animal system ***[F2]*** with free access to food and water.

In Vivo Delivery of Nicotine via the Probe: The in vivo relative delivery of nicotine by the probe was determined using a perfusion fluid containing 40 µM nicotine in Ringer's solution. To evaluate probe behavior over time, the delivery experiment was first conducted 3 to

2701 Kent Avenue West Lafayette, IN 47906 765-463-4527 fax 765-497-1102 basi@bioanalytical.com
www.bioanalytical.com

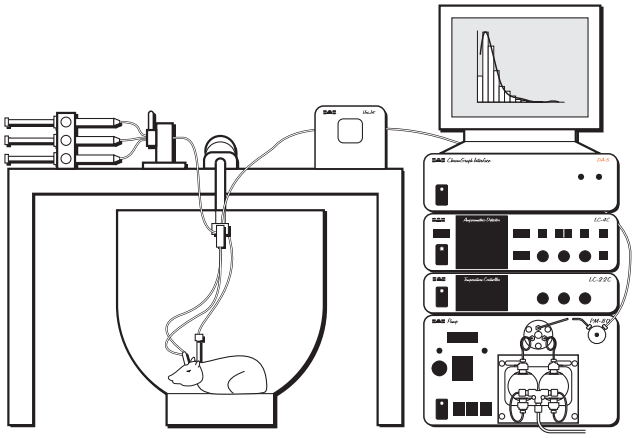

Figure 2. *BAS BeeKeeper awake animal system showing microdialysis sampling coupled on-line to a liquid chromatography system.*

4 hours after surgery (day 0) and repeated daily. The relative delivery, D, was calculated according to the following equation:

$$D = C_i - C_d / C_i = 1 - (Cd/C_i)$$

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.

Nicotine Dose via the Patch: After 3 hours of baseline sampling, during which no interfering peaks appeared, a section of dermal patch (Nicotrol,® McNeil Consumer Products Co., PA) containing ca. 2 mg nicotine was affixed to the dermis above the implanted probe. The full size patches intended for human use were cut into smaller pieces to obtain an appropriate dose for use with the rats. In cutting the patch, we assumed uniform distribution of the nicotine across the area of the patch. The estimated average dose was 5 mg/kg/24 hours. Dialysate samples were automatically injected into the LC system at 8 minute intervals for the first 5 hours and at 30 minute intervals for the rest of the experiment, up to 24 hours. The concentration of nicotine in the dialysate was calculated for a standard curve.

Results and Discussion

In Vivo Delivery: The results of daily in vivo delivery of nicotine via the probe is shown in ***F3***. Except for the initial, day 0, delivery the values are fairly uniform. Changes in circulation as an initial response to probe implantation 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 [1]. After implanting a probe in the forearm skin of human subjects, they observed 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 modulated the time frame for circulatory changes. While the data presented in ***F2*** show gradual decreases in delivery from days 1 through 3, the deliveries on days 4 and 5 increase to

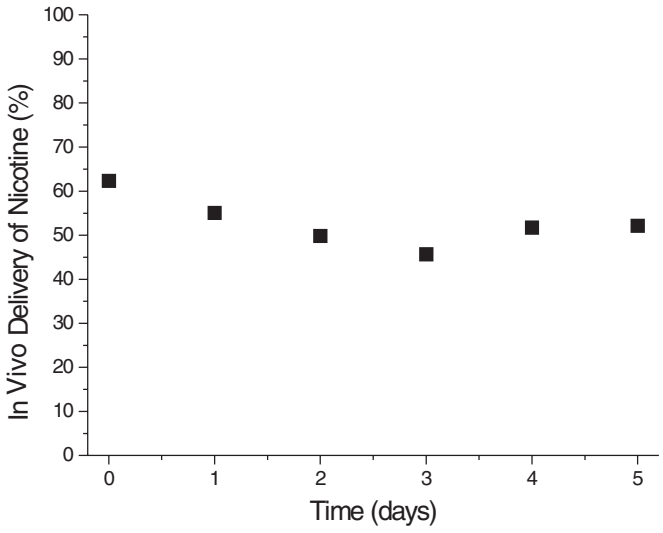

Figure 3. *In vivo delivery of nicotine via a linear microdialysis probe implanted in the dermis of a rat. Points are average relative delivery based on at least four consistent sequential sample injections.*

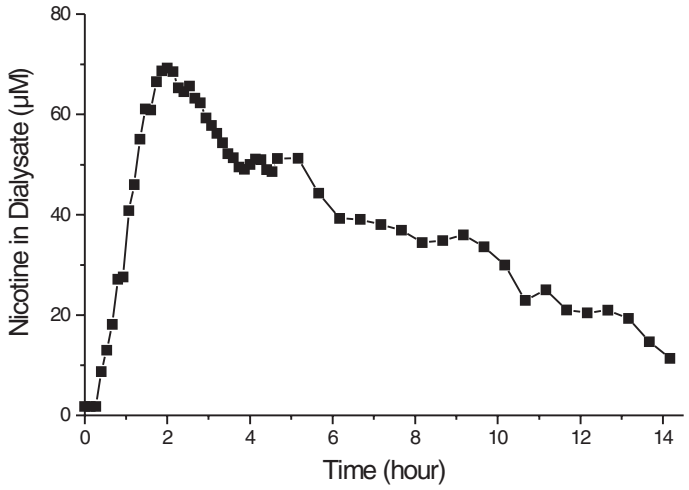

Figure 4. *Typical concentration time profile of nicotine as monitored by a linear microdialysis probe in the dermis of an awake rat. A patch containing ca. 2 mg nicotine was applied above the probe membrane window.*

about the level observed on day 1. Overall the probe behavior was consistent throughout the week.

F4 shows a typical temporal profile of nicotine concentration in the extracellular fluid of the skin of a rat. Nicotine was first detected in the dialysate 40-60 minutes after placing the patch on the skin. The peak level was reached about 2 hours after application. As can be seen in ***F4***, the nicotine levels in the dermis remain relatively stable for several hours before slowly declining in a stepwise fashion. The patch manufacturer's specification indicated steady delivery of the nicotine for 16 hours following application. The plateau is not as level or as long as might be expected. Inconsistent contact between the skin and patch could account for the dips in the plateaus.

This research demonstrated 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.

References

1. Anderson, C, Andersson T, and Wårdell K, Changes in skin circulation after insertion of a microdialysis probe visualized by laser Doppler perfusion imaging. *Journal of Investigative Dermatology* **102**: 807-811, 1994.

For additional information concerning microdialysis sampling in skin, see:

Andersson T., Wårdel K, and Anderson C, Human in vivo cutaneous microdialysis: estimation of histamine release in cold urticaria. *Acta Derm Venereol* **75**: 343-347, 1995.

Ault JM, Lunte CE, Meltzer NM, and Riley CM, Microdialysis sampling for the investigation of dermal drug transport. *Pharmaceutical Research* **10** (9): 1256-1261, 1992.

Ault JM, Riley CM, Meltzer NM, and Lunte CE, Dermal microdialysis sampling *in vivo. Pharmaceutical Research* **11**(11): 1631-1639, 1994.

Hegemann L, Forstinger C., Partsch B, Lagler I, Krotz S, and Wolff K, Microdialysis in cutaneous pharmacology: Kinetic analysis of transdermally delivered nicotine *Journal of Investigative Dermatology* **104** (5): 839-843, 1995.

Müller M, Schmid R, Wagner O, Osten B, Shayganfar H, and Eichler HG, In vivo characterization of transdermal drug transport by microdialysis. *Journal of Controlled Release* **37**: 49-57, 1995.

Müller M, Osten B, Schmid R, Piegler E, Gerngroβ I, Buchegger H, and Eichler HG, Theophylline kinetics in peripheral tissues in vivo in humans. *Naunyn-Schmeideberg's Archives of Pharmacology* **352**: 438-441, 1995.

Peterson, LJ, Nielsen HJ, and Skov PS, Codeine-induced histamine release in intact human skin monitored by skin microdialysis technique: comparison of intradermal injections with atraumatic intraprobe drug delivery system. *Clinical and Experimental Allergy* **25** (11): 1045-1052, 1995.