Pharmaceutical Research, Vol. 6, No. 11, 1989

Technical Note

Noninvasive Sampling of Biological Fluids by Iontophoresis

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Received June 29, 1988; accepted May 30, 1989
KEY WORDS: noninvasive sampling; iontophoresis; skin; glucose monitoring.

Medical diagnosis and in-patient drug monitoring rely upon the detection and quantitation of endogenous and exogenous bioactive chemicals. Currently, such analysis is predominantly based upon blood sampling which is achieved invasively via needle. The inconvenience and limitations of this procedure are well-known: patients, on the whole, would rather not be injected, the frequency and amount of sampling is constrained, successful intravenous access in (for example) pediatric and geriatric patients may be difficult, and there are risks to both patient (e.g., infection) and sampler (e.g., exposure to pathogen-contaminated blood). The potential benefits of alternative, noninvasive sampling procedures for chemical exposure assessment and continuous drug monitoring have been described (1–3). One approach (1,3) involves collection, via the skin, of molecules circulating systemically. A major difficulty, however, with this idea is the characteristically very slow and variable passive permeation rates of chemicals across the skin (4). Indeed, experiments examining the outward migration of theophylline revealed little correlation between sampled amounts and drug levels in the body (3). Additionally, it was necessary to collect samples over extended periods of time, a significant potential drawback.

To circumvent these problems, the application of iontophoresis to enhance sampling efficiency (rate and extent) has been examined. Iontophoresis employs an electrical potential gradient to promote the penetration of (typically) ionizable molecules across (usually) the skin (5). The current uses of the technique are the treatment of hyperhidrosis (6) and the diagnosis of cystic fibrosis (7), in which pilocarpine is iontophoresed into the skin to induce sweating. There is considerable research activity, furthermore, into transdermal iontophoretic drug delivery as an alternative to conventional administration routes (5,8,9). The approach has attracted particular interest with respect to the delivery of peptides (8) and small proteins (10).

In the experiments described here, advantage has been taken of the symmetry of the iontophoresis procedure: namely, that, just as delivery can be enhanced by the imposition of a potential gradient, so can the reverse "back-extraction" process. Initial studies have been conducted *in vitro* using a novel diffusion cell (Fig. 1), specifically designed for iontophoresis research (11). The cell allows both cathode and anode to be applied to the same side of a single, continuous piece of skin (mimicking, thereby, the situation which exists *in vivo*). Electrode compartments are electrically and physically isolated from one another in this configuration. The model membrane employed to separate the upper and lower halves of the diffusion cell was full-thickness skin taken immediately post-sacrifice from hairless mice (Skh:HR-1, 8–13 weeks old). The electrodes consisted of self-adhesive gels (modified Kenzgelelc, Nitto Electric Industry Co. Ltd., Osaka, Japan) into which platinum wires were inserted. The electrodes were connected to a power supply (APH 1000M, Kepco Inc., Flushing, NY) which was set to deliver a constant current of 0.5 mA. The lower receptor compartment of the cell was perfused (at 10 ml/hr) with radiolabelled permeant solutions prepared in pH 7.4 phosphate-buffered saline. Following assembly of the cell, and initiation of perfusion, current was applied for 2 hr. At the end of this time, the electrodes were disconnected and radioactivity in the gels was determined by liquid scintillation counting. Figure 2 presents the results (i.e., amount of permeant "absorbed" into the cathodic gel), when the receptor chamber was perfused with solutions of (a) [^{3}H]clonidine hydrochloride (sp act = 24.1 Ci/mmol; purity > 95%) and (b) [^{14}C]theophylline (sp act = 38 mCi/mmol; purity > 95%), at various concentrations. Each data point is the mean of between two and five separate experiments. We observe the following.

(1) Despite the brevity of electrical current application, the amounts of drug accumulated (or sampled) are sufficient that non-isotopic analytical procedures could have been used.
(2) If no current was applied, then no radioactivity was found in either gel electrode following perfusion for a comparable period.
(3) A linear correlation was obtained between drug concentration in the perfusate and the amount extracted iontophoretically.

Subsequently, we examined whether enhanced iontophoretic sampling could also be applied to uncharged permeants. Recent reports in the literature (8,12) have shown that the transport of polar, but *neutral*, compounds can be substantially promoted by the imposition of a potential gra-

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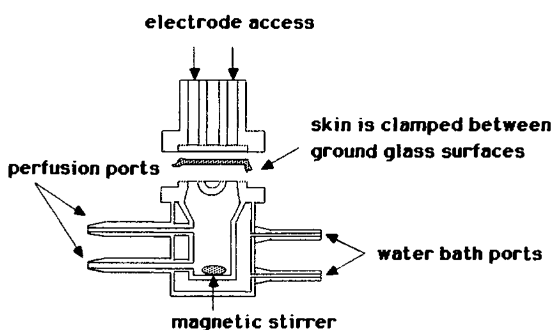

Fig. 1. *In vitro* iontophoretic diffusion cell (11). The vertical walls in the top compartment are aligned with the edges of the trough traversing the upper portion of the bottom compartment. With the skin positioned as shown (epidermal surface up), the electrodes are physically and electrically isolated from one another. As lateral current flow within the skin appears to be negligible (11), the design of the cell is a reasonable mimic of iontophoretic use *in vivo*. The overall diameter of the cell is 2.5 cm. The area of skin surface exposed in each electrode compartment is 0.8 cm^2.

dient across the skin. The precise mechanism by which this phenomenon occurs is not completely understood but is thought to involve a convective or electroosmotic effect, whose origins lie in the apparent permselectivity of the skin to positive ions (13). Sodium ions, in particular, have a relatively high mobility across the skin and are believed to induce the flow of neutral species when a current is passed. This has been elegantly demonstrated by Burnette and Ongpipattanakul (12,13) using mannitol as the model, neutral permeant, the passive flux of which was considerably enhanced. In terms of percutaneous "extraction," we chose to examine glucose, a molecule for which noinvasive sampling would provide benefit to diabetics. *In vitro* experiments were performed as described above, with the receptor chamber perfused now with solutions of glucose in pH 7.4 phosphate-buffered saline. In a first series of experiments, glucose concentrations of 0.153 and 1.07 mg/ml (levels lower than those typically found in the blood of a nondiabetic) were perfused for 2 hr, during which time a constant iontophoretic current of 0.5 mA was flowing. Glucose extraction was monitored at the cathode, analysis of the gel being facilitated by the incorporation of [^{14}C-U]glucose (sp act = 325 mCi/mmol; purity >99%) into the perfusing solution. The results in Table I show that the ratio of perfused concentrations (0.143) was equal to the ratio of extracted glucose (0.144). Further, if no current flowed, glucose could not be detected in the electrode compartments. Next, the temporal pattern of glucose extraction was studied. In this case, a radiotagged glucose solution (0.34 mg/ml) was perfused for 2.5 hr, and the cathodic gel electrode was assayed every 30 min (by exchanging the previous gel for a new one). The data (Table II) show that a reasonably constant amount (about 0.8 μg) of glucose is extracted in each half-hour. However, the coefficient of variation in this measurement is high (23%). Table III presents the individual data from each diffusion cell employed and indicates that the variability is specifically attributable to the results from cell 3. No obvious explanation for the scattered and relatively low results from this cell is apparent, although imperfect, intermittent electrical contact somewhere in the iontophoretic circuit could be a possible cause. The results in Table III do not include the first sample obtained in the 0- to 0.5-hr period. Because of the mechanics involved in setting up a battery of iontophoretic cells, the skin has an opportunity to rest in contact with the glucose solution for up to an hour before the current is applied. Due to this experimental (though perhaps not actual) artifact, the values of the initial samples are not presented.

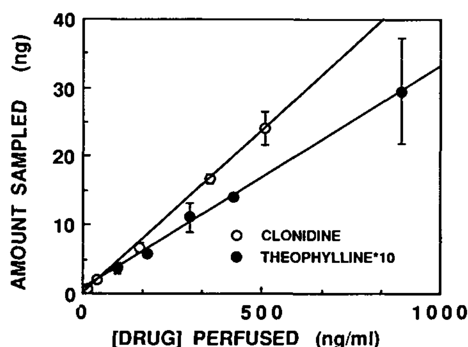

Fig. 2. *In vitro* iontophoretic sampling of clonidine and theophylline across hairless mouse skin. Various drug concentrations were perfused through the receptor (bottom) chamber of the diffusion cell (Fig. 1) for 2 hr. The amounts of drug extracted into the cathodic gel on the skin surface in the upper chamber are linearly correlated (r = 1.00) with the subcutaneous circulating drug level.

Table I. Iontophoretic Sampling (Mean ± SD; n = 4) of Glucose Across Hairless Mouse Skin *in Vitro*

[Glucose] (mg/cm^3) perfused subcutaneously[a]	Amount (μg) of glucose sampled[b] in 2 hr
0.153[c]	0.71 ± 0.10[d]
1.07[c]	4.90 ± 0.70[d]

[a] Perfused at 10 cm^3/hr.
[b] Sampling performed in the aqueous gel surrounding the cathode on the skin surface.
[c] Ratio of perfused concentrations = 0.143.
[d] Ratio of amounts sampled = 0.144; coefficient of variation = 14%.

In summary, a novel, potentially noninvasive sampling procedure is described. The technique gives a linear response *in vitro* and suggests that the extraction of bioactive materials from the body may be possible. Theoretically, the efficiency of sampling depends upon the current applied and

Table II. Iontophoretic Glucose Sampling[a] Across Hairless Mouse Skin *in Vitro* as a Function of Time (Mean ± SD; n = 4)

Time period (hr)	Amount (μg) of glucose sampled
0.0–0.5	0.97 ± 0.12
0.5–1.0	0.79 ± 0.09
1.0–1.5	0.76 ± 0.10
1.5–2.0	0.75 ± 0.21
2.0–2.5	0.74 ± 0.24
Average	0.80 ± 0.19[b]

[a] Concentration of glucose perfused subcutaneously at 15 cm^3/hr = 0.34 mg/cm^3.
[b] Coefficient of variation = 23%.

Table III. Intersample Variability in Iontophoretic Glucose Sampling Across Hairless Mouse Skin *in Vitro*

Cell No.	Amount of glucose (μg) per 0.5-hr sample[a]
1	0.83 ± 0.04 (5%)[b]
2	0.96 ± 0.09 (9%)
3	0.53 ± 0.12 (22%)
4	0.73 ± 0.03 (4%)

[a] Mean ± SD ($n = 4$) of samples taken in four half-hourly periods between 0.5 and 2.5 hr post initiation of iontophoresis.
[b] Values in parentheses are coefficients of variation.

the duration of current flow, and these parameters can be carefully and precisely manipulated. Considerable further work is necessary fully to validate the approach and to test the above hypotheses.

ACKNOWLEDGMENTS

We thank M. Hori and Nitto Electric Industrial Co. Ltd., Osaka, Japan, for the gel electrodes. This work was supported by the National Institutes of Health (HD-23010), Cygnus Research, and Syntex Research, Inc.

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