Pharmaceutical Research, Vol. 5, No. 7, 1988

Report

A New System for *In Vitro* Studies of Iontophoresis

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Received August 28, 1987; accepted February 6, 1988

This report describes a new iontophoretic diffusion cell that allows both electrodes to be applied to the same side of the same piece of skin. The cell permits a better approximation of the *in vivo* situation than do conventional side-by-side cells. The unique construction of the cell allows nonliquid material to be applied to the skin surface and makes it possible to investigate horizontal transport paths. Preliminary results utilizing the cell are described. Iontophoretic enhancement of morphine and clonidine delivery across full-thickness hairless mouse skin has been achieved. The importance of pH control in these experiments is apparent. Further experiments with morphine indicate that, for this drug at least, iontophoretically driven lateral transport within the skin is unimportant. Because the cell design allows significant parallels to the use of iontophoresis *in vivo*, we suggest that it will prove to be a useful tool in the determination of fundamental structure/transport relationships under the influence of an externally applied current.

KEY WORDS: iontophoresis; diffusion cell; transdermal drug delivery.

INTRODUCTION

Iontophoresis is the electrically enhanced transport of bioactive materials. Although it is well-known as a means of transdermal drug delivery (1–3), its mode of operation is not well understood. A typical *in vivo* iontophoretic system consists of two electrodes which are placed on the outside of the skin and are connected to a power supply. The bioactive material is placed in a conductive medium between the "active" electrode and the skin. A conductive medium between the second ("passive") electrode and the skin completes the circuit. When current flows throught the circuit, the bioactive material is transported into the skin.

In the standard arrangement for *in vitro* iontophoretic studies, the two halves of a diffusion cell are placed side by side so that the skin is located vertically between them, with its epidermal side facing one half and its inner side facing the other. The bioactive preparation and the active electrode are put in the "epidermal" half of the cell, and the other side of the cell contains the passive electrode in a conductive fluid.

This side-by-side arrangement has several drawbacks and limitations. Since the passive electrode is, in effect, placed "inside" the skin, this configuration is not a good model of the *in vivo* case. The factors that influence such a nonphysiological situation may not be those that are important in the clinical case. In addition, there are questions that cannot be investigated with a side-by-side configuration, such as the possibility of horizontal transport (i.e., between skin layers rather than through the skin) and whether an iontophoretically driven drug is being "pulled" back out of the skin by the passive electrode.

MATERIALS AND METHODS

Description of the Cell. This new iontophoretic diffusion cell is constructed so that one half of the cell is above the other half. The excised skin is interposed horizontally, with the epidermal surface (area, 0.8 cm^2) interfaced with the upper half of the cell (see Fig. 1). The upper half of the cell is divided by two vertical walls into three chambers. The outer two chambers are therefore separated by an intervening space (the third chamber). The lower half of the cell holds the collection fluid (volume, ~7 cm^3). The walls that form the middle chamber in the upper half are continued into the lower half of the cell but are then joined to form a small channel which traverses the top of the lower half of the cell.

When the upper half of the cell is positioned over the lower half so that the upper and lower walls coincide, the strip of skin between the walls is sealed off from the skin in the electrode chambers on both its upper and its lower sides. The portions of skin in the electrode chambers are thus physically and electrically isolated from each other so that the flow of current and biomaterial through and within the skin can be investigated. Ports in the lower half-cell allow fluid to be continuously collected. The flow rate is set to maintain "sink" conditions through an experiment. During a transport study, the channel at the top of the lower half of the cell is filled with collection fluid so that the underside of the skin remains moist. The walls of the channel also provide mechanical support for the skin.

The diffusion cells were made by Skin Permeation Systems (LGA, Berkeley, Calif.). The cell is a modification of a standard flow-through design (LGA skin penetration cell, catalog No. LG 1084-MPC), described by Gummer *et*

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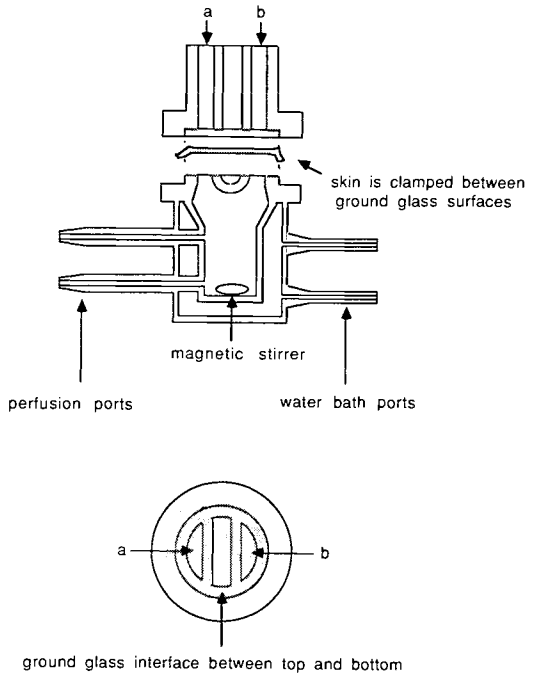

Fig. 1. Iontophoretic diffusion cell. The cell is constructed of glass. The effective skin surface area is 0.8 cm^2. The height of the upper half of the cell is 0.5–2.5 cm. Typically, the donor phase (solution, gel) has a volume of about 0.5 cm^3. The volume of the receptor phase is approximately 7 cm^3.

al. (4). In use, the space between the electrode chambers (a and b in Fig. 1) in the upper half of the cell decreases the possibility of leakage and makes it possible to investigate questions involving skin continuity. The bottom half of the cell, in addition to the two ports for the continuous flow of the receptor phase, also has two ports for water jacket circulation. Capillarity between the compartment walls and the external well was prevented by silanizing the top of the cell with dichlorodimethyl-silane (Aldrich Chemical Co., Milwaukee, Wis.). The cells were used with a three-station magnetic stirrer unit (LG-1083-MS, LGA, Berkeley, Calif.).

Electrodes. Platinum wire (Pt wire, Fisher No. B-766-5A, 99.95% pure) was immersed directly in solutions contained in chambers a and b. "Gel" electrodes (Kenzgelelc, Nitto Electric Industry Co. Ltd., Osaka, Japan) were used, in which the polymer forms an electrical bridge between the Pt wire and the skin.

Power Supply. Current and voltage control with automatic crossover (Model APH 1000M, Kepco, Inc., Flushing, N.Y.) was used. This supply has a specified drift of <2 μA/8 hr for its current-controlled output, an important consideration if drug flux is sensitive to changes in current (3,5).

Receptor Fluid. The receptor fluid was phosphate-buffered saline (pH 7.4; 0.9% NaCl, w/v).

Dye. Blue dye No 1. FD&C in deionized water was used.

Drugs. The following drugs were used: clonidine-HCl (Sigma Chemical Co., St. Louis, Mo.), clonidine-HCl (phenyl-4-^3H) of specific activity 90 mCi/mg (Amersham, Arlington Heights, Ill.), morphine sulfate (Sigma Chemical Co., St Louis, Mo.), and morphine (*N*-methyl-^3H) of specific activity 255 mCi/mg (New England Nuclear, Boston, Mass.). The "cold" drugs were dissolved in deionized water to form solutions of 1 mg/ml, and labeled drug was added to achieve an activity of approximately 1 μCi/ml.

Skin. Full-thickness skin, freshly excised from 11- to 15-week-old female hairless mice (strain Skh:HR-1, Simonsen Laboratory, Gilroy, Calif.), was used.

Evaluation of the Cell. The diffusion cell was tested in three ways: (1) leakage tests (without current) using dye and silicone rubber rather than skin, (2) leakage tests using dye and skin (without current), and (3) iontophoretic tests using the drug solutions and skin (with and without current). Procedures 1 and 2 were evaluated by visually inspecting the cell. For procedure 3, 0.6 ml of labeled drug solution was placed in chamber a, 0.6 ml of buffered saline was pipetted into chamber b, and a constant current of 0.5 mA (with the voltage limited to 9 V) was imposed between the electrodes in the two chambers. The activity of the solutions in chambers a and b was determined before and after iontophoresis. The activity of the skin and of the samples taken from the receptor chamber was determined postexperimentally. Each experiment lasted approximately 24 hr, with samples collected hourly. The receptor fluid was magnetically stirred, and the collection flow rate was 10 ml/hr. Each procedure was repeated in triplicate.

RESULTS

Procedures 1 and 2. No dye leakage was observed from the side chambers to the middle chamber, or from any chamber to the outside of the cell, for both the model silicone rubber membrane and the hairless mouse skin.

Procedure 3. When the dye in chamber a was replaced with labeled drug and no current was applied, the drugs diffused into the receptor phase with mean rates of 0.05 μg/cm^2/hr for clonidine-HCl and 0.04 μg/cm^2/hr for morphine sulfate. In both cases, no drug was found in the buffered saline of chamber b after 20 hr.

When current was applied between the chamber with the labeled drug and the chamber with the buffered saline, permeation increased substantially. For morphine sulfate, iontophoretic transport was examined using a number of different "vehicles" in chamber a (Fig. 2). Two initial runs, for

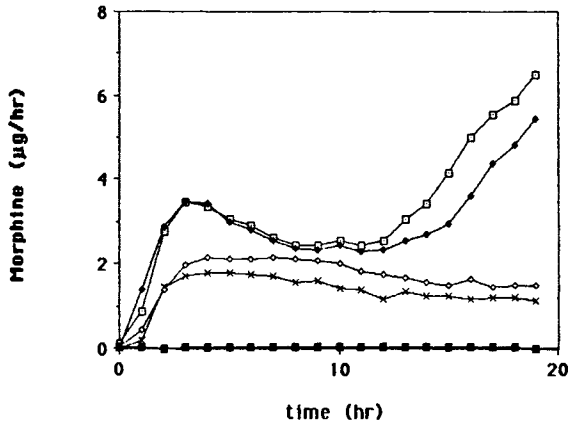

Fig. 2. Transdermal delivery of morphine sulfate. Passive permeation (■) is compared with iontophoretic transport from unbuffered aqueous solution (□, ◆), solution buffered at pH 5 (×), and solution buffered at pH 7 (◇).

which the drug solution was not buffered, resulted in the pH of chamber a falling from above 6 to ca. 1.5. Deterioration of the skin barrier is apparent once the experiment duration exceeds 10 hr. Buffering of the donor phase at pH 5 or pH 7 resulted in transport that became essentially zero order after 3 hr. The flux at this time was about 2 μg/cm²/hr, i.e., approximately 50 times the passive (no-current) rate. If the electrode was surrounded by a simple, aqueous buffered gel, morphine delivery was again ca. 2 μg/cm²/hr. In the "buffered" experiments, radiolabeled morphine was detected in the saline in chamber b; approximately 1 μg was present after 20 hr.

For clonidine-HCl, using fully buffered vehicles (solutions and gels), the maximum flux with iontophoresis was between 12 and 28 μg/cm²/hr, i.e., an enhancement of 240- to 560-fold (Fig. 3). The maximum rate was typically attained by 4 hr, but constant delivery was not maintained for a prolonged period due to depletion of the drug in the donor phase. As for morphine, labeled clonidine was detectable in chamber b: ca. 5 μg was present after 20 hr.

It is apparent from Fig.2, for example, that comparison of "lag times" between passive and iontophoretic transport is difficult. With current, drug is always detected in the sample collected during the first hour of the experiment. More samples would have to be taken during the initial 3–4 hr of iontophoresis to determine a reliable lag time. In the absence of current, drug flux is very small and, again, precise determination of lag time is difficult.

DISCUSSION

The results in Figs. 2 and 3 demonstrate that the new diffusion cell (Fig. 1) provides an adequate means to induce and monitor iontophoretic enhancement of percutaneous penetration. The design of the cell is such that a relevant *in vitro* model of the actual *in vivo* situation is now available.

The appearance of both morphine and clonidine in chamber b following iontophoresis is intriguing. The initial testing of the cell would seem to rule out leakage. However, from the experiments presented so far, it is impossible to say whether horizontal transport through the skin or "extraction" of drug from the receptor phase by the "passive" electrode is responsible for the observation. To address this unknown, two cells were connected so that their receptor phases were in common but the skin and cell tops were physically separated (Fig. 4). Labeled morphine sulfate solution, buffered at pH 5, and the positive electrode were positioned in chamber a of cell I. The cathode was placed into chamber b of cell II. All other chambers were filled with buffered saline. The remaining experimental procedures were identical to those of procedure 3 (see above). With this configuration, labeled drug *was* detected in chamber b of cell II, at levels comparable to that found in the single-cell experiments (i.e., about 1 μg after 20 hr). Obviously, leakage and lateral skin transport cannot occur in the double-cell system, and it follows that morphine was indeed "extracted," via reverse iontophoresis, by the "passive" electrode.

It should also be pointed out that the configuration in Fig. 4 did not appear to alter the amount of morphine iontophoresed into the receiver compartment. The data in Fig. 5 compare morphine delivery kinetics in the "single-cell" and "double-cell" configurations. Although current was applied for only 6 hr (i.e., long enough to achieve maximum drug flux under these conditions), there was no difference in transport rates. The only inconsistent parameter was the

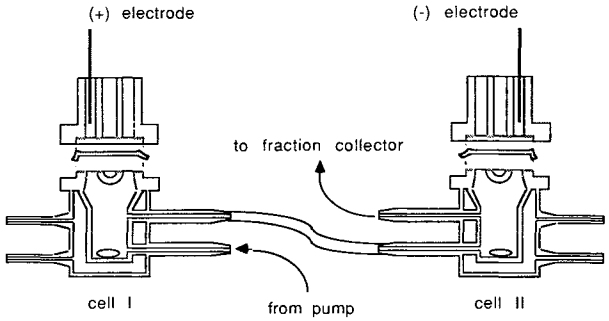

Fig. 4. Double-cell connections.

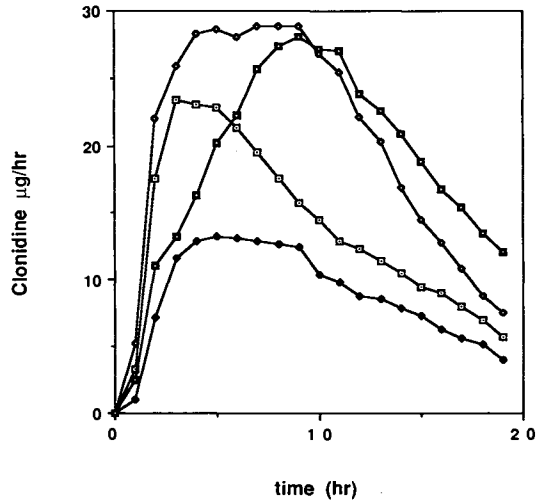

Fig. 3. Transdermal delivery of clonidine-HCl. Iontophoretic transport from buffered solution (□, ◆) and aqueous buffered gel (■, ◇).

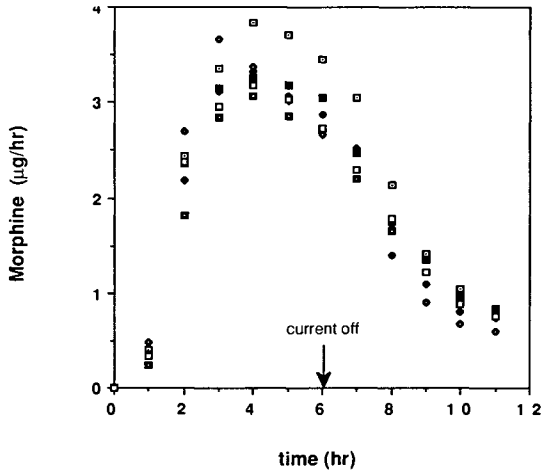

Fig. 5. Morphine sulfate delivery from buffered solution in a single-cell system (filled symbols) is compared with that in the double-cell configuration (open symbols) depicted in Fig. 4. The current was applied for 6 hr and then discontinued.

voltage applied between the electrodes to maintain the current constant at 0.5 mA. Because of the extra receptor fluid and tubing in the double-cell setup (Fig. 4), the total resistance of the system is greater. A larger voltage (by ~50%) was therefore necessary to hold the current at the desired level.

Overall, these experiments show, first, that significant lateral morphine transport does not take place and, second, that the "passive" electrode can extract drug, which has been iontophoresed through the skin by the active electrode, back across the barrier. This discovery has implications both for the optimal placement of the passive electrode in a therapeutic device and for the possibility of iontophoretically sampling the subcutaneous space.

It is well-known that the electrically driven flow of ions across a membrance can induce a coupled flow of solvent molecules (6). Such electroosmotic flow may have been observed in this system. With no current flowing between the electrodes, and phosphate-buffered saline or aqueous drug solution in chambers a and b, there was no change in volume in either chamber. However, when 0.5 mA of current was applied between the electrodes (anode in a, cathode in b), a reduction (25%) in volume occurred in chamber a, whereas the volume in chamber b *increased* by 17%. The high transport number of Na^+, the major anion present, probably accounts for this result. To pursue these observations further clearly requires more detailed and careful experimentation.

In summary, a unique diffusion cell for iontophoretic studies has been designed and tested. A crucial feature of the cell, with respect to its relevance to the *in vivo* situation, is that both electrodes can be positioned on the outer surface of a single, continuous piece of skin. The cell has allowed the significance of lateral drug transport through skin to be evaluated. Further, the cell permits vertical access to the skin surface for the application of drugs in paste, gel, or other nonliquid forms; this versatility cannot be achieved using conventional side-by-side cells. Considerable exploration of the cell in basic research investigating the phenomenon of iontophoresis is anticipated.

ACKNOWLEDGMENTS

This work was supported in part by NIH Grants GM-33395 and HD-23010 and by Cygnus Research. Christopher Cullander is the recipient of a fellowship from the Biotechnology Research and Education Program of the University of California.

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