Electrically Modulated Transdermal Delivery of Fentanyl

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Received November 29, 2001; accepted December 10, 2001

Purpose. Test to determine if iontophoresis and electroporation, alone or in combination, can be used for rapid and modulated delivery of fentanyl.

Methods. Fentanyl citrate (5 mg/ml) dissolved in pH 4.0 citrate buffer was delivered *in vitro* across human epidermis. For iontophoresis, a current of 0.5 mA/cm^2 was applied for 5 h, using silver/silver chloride electrodes. Electroporation protocol consisted of applying 15 exponential pulses of 500V (applied voltage) and 200 msec duration at the rate of 1 pulse per minute at time zero and, in some cases, repeating at 1.5 and 2.5 h.

Results. There was no measurable permeation of fentanyl through human epidermis under passive conditions. A significant flux (about 80 μg/cm^2-hr) was achieved using iontophoresis and decreased once the current was turned off. A 4-fold higher flux and shorter lag time was observed with electroporation as compared to iontophoresis. The flux was found to recover quickly (within 1 h) following pulsing. Modulation of transdermal delivery of fentanyl was demonstrated by both iontophoresis and electroporation.

Conclusions. Electrically assisted transdermal delivery of fentanyl significantly increased transport compared to passive delivery. Also, rapid and modulated delivery was shown to be feasible by programming the electrical parameters.

KEY WORDS: transdermal; iontophoresis; electroporation; fentanyl.

INTRODUCTION

Fentanyl is a potent synthetic opioid widely used as an analgesic and as a narcotic analgesic supplement in general or regional anesthesia due to its rapid onset, short duration of action and high potency (80 times more potent than morphine). Due to the extensive first-pass metabolism of fentanyl, it is available as parenteral and transdermal dosage forms. Controlled delivery is desirable and transdermal delivery of fentanyl is now widely used (1–4). A fentanyl transdermal system (Duragesic®, Janssen, J&J) was approved for marketing in the United States in 1991. Currently, it is the only narcotic analgesic commercially available in a transdermal dosage form. The marketed Duragesic® patch delivers fentanyl at a rate of 25, 50, 75, or 100 μg/h from four dosage strengths (Physicians' Desk Reference 2001, 55th Edition, Medical Economics Co., Montvale, NJ). It is approved for the management of chronic pain, especially cancer pain. The patch delivers fentanyl at a controlled rate for 3 days upon application, providing effective pain relief during this period. Fentanyl appears in blood within a few hours of applying the patch, with consistent steady-state serum levels achieved within 12–24 h and maintained over the remaining 2 days (5). Transdermal fentanyl delivery from Duragesic® has a slow onset of action and a long duration. Therefore, efforts are underway to develop a patch with a faster onset and shorter duration of action for the control of postoperative pain (6–8). Currently, transdermal fentanyl can only be used for treatment of chronic pain in patients who require continuous long-term management. However, its application in treating cancer pain offers an alternative to oral morphine as it has fewer gastrointestinal side effects (9).

The development of an iontophoretic patch with a faster onset of action may allow treatment of acute pain, e.g., postoperative pain since individualization and titration of dose may become feasible by changing electronic parameters. This titration of dose to individual patients' demand is desirable (10). It may be accomplished by iontophoretic delivery that provides noninvasive, active transdermal delivery by application of a low-level of electrical current (0.1–0.5 mA/cm^2 of skin) over periods of minutes to hours (11,12). Clinical trials have shown that therapeutically significant serum fentanyl concentrations (1–3 ng/ml) can be attained by use of iontophoresis. Analgesic doses of fentanyl were administered by iontophoresis for delivery periods of up to 2 h. Mean times to detectable fentanyl plasma concentration were 33 and 19 min for 1 and 2 mA deliveries, with corresponding maximum concentrations being 0.76 and 1.59 ng/ml after 122 and 119 min, respectively (13). A wearable iontophoretic patch (E-TRANS®) for delivery of fentanyl is currently under commercial development (14). Clinical evaluation on the pharmacokinetics and safety of fentanyl delivery by the E-TRANS® system has been reported (15,16). The application of a basal current of 100 μA for 26 h provided sufficient concentration of fentanyl in serum to produce effective analgesia.

Electroporation has been widely used to provide rapid infusion of drug molecules, especially macromolecules across the stratum corneum, the outermost layer of the skin (17,18). Skin electroporation results in the transient permeabilization of the stratum corneum by creating new aqueous pathways, or pores using short (μsec to msec), high voltage electrical pulse. These pores allow drug molecules to efficiently enter in the skin. Thus, the mechanism/pathway is different from that in iontophoresis where existing transappendageal pathways (hair follicles and sweat glands) are utilized for transport. *In vitro* and *in vivo* delivery of fentanyl by electroporation has been investigated (19,20). This report explores the strategy of combined use of iontophoresis and electroporation for a synergistic delivery of fentanyl through skin. More importantly, for the first time, we were able to achieve a fast onset of permeation and a modulated delivery profile using electroporation.

MATERIALS AND METHODS

Materials

Fentanyl citrate was purchased from Sigma (St. Louis, MO). Other chemicals were purchased from Fisher Scientific (Pittsburgh, PA). Human cadaver skin was obtained from the

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0724-8741/02/0400-0440/0

National Disease Research Interchange (Philadelphia, PA). The skin was received on dry ice and stored frozen at –80°C until ready for use.

Transdermal Transport Studies

Full thickness human cadaver skin was used to separate the epidermis by the widely used heat treatment method. Briefly, human epidermis was prepared by heating full thickness human skin in water at 60°C for 45 s, and rubbing gently with two broad spatulas. The epidermal membrane was then teased off the underlying dermis with forceps and mounted on Franz transdermal diffusion cells. An external water bath maintained the temperature of the circulating water in the jackets at 37°C. Experiments were performed in triplicate. The donor solution consisted of fentanyl citrate (5 mg/ml) dissolved in pH 4.0 citrate buffer (100 mM) containing 75 mM sodium chloride. Fentanyl has a pK_a of 8.9 and is almost 100% ionized at the donor pH. The receptor solution was pH 7.4-phosphate buffer with 75 mM sodium chloride. Samples were taken from the receptor compartment and analyzed by a HPLC assay. For iontophoresis, a constant current of 0.5 mA/cm^2 was applied using a Dupel® (Empi, Inc., MN) device for a period of 5 h, using a silver wire as the anode in donor and a silver/silver chloride electrode as the cathode in the receptor. The electroporation protocol consisted of applying 15 pulses at the rate of 1 pulse per minute at time zero and for some experiments, repeating the protocol at specified time intervals. The applied pulse voltage was 500 V and pulse length was 200 msec, applied via an exponential pulse generator ECM 600 (Genetronics, Inc., San Diego, CA). We have previously found this protocol to result in optimal transdermal delivery for other drugs (21). The results of the permeation experiments will be plotted as flux or cumulative amounts of drug permeated vs. time. Samples were replaced with the receptor medium and this was taken into consideration in the calculations that were done on spreadsheet software. Statistical analysis was performed using single factor ANOVA.

HPLC Assay

A modified HPLC assay for fentanyl was used with a 25 cm 5 micron C-8 column (22). The mobile phase was composed of aqueous perchloric acid (0.23%) and acetonitrile (65:35) and detection was performed at a UV wavelength of 206 nm. The assay was found to be linear over the range of 20 to 100 μg/ml, with a correlation coefficient of 0.9999, percent Y-intercept of less than 1.5%, and a relative standard deviation between samples of less than 1.8%. The lower limit of quantitation of fentanyl was 750 ng/ml. The retention time for fentanyl was approximately 12 m.

Skin Impedance Studies

The electrical properties of the skin prior to iontophoresis and electroporation were measured *in vitro* using a function generator (V_0 = 1V; repetition at f = 1KHz; sine wave). The resistance of the chamber without the skin, R_{Bulk}, was also measured in a similar fashion. The skin was pulsed using an exponential pulse generator ECM 600 (Genetronics, Inc., San Diego, CA). To measure the applied voltage (V_0), a Tektronic digital oscilloscope was used and the trace stored in channel 1. The voltage developed across the 15Ω resistor in series with the chamber was measured at channel 2 (V_s). This would give the total current through the chamber during an electroporation pulse. The effective transdermal voltage across the stratum corneum (V_{Skin}) was calculated using the equation,

$$V_{Skin} = V_0 - V_s^* (1 + R_{Bulk}/15) \quad (1)$$

The electrical transport efficiency of iontophoresis and electroporation can be compared by the amount of drug transported with respect to total charge delivered across the skin. The total charge (coulombs) delivered across the skin by iontophoresis may be defined as the applied current (I_a) in amperes multiplied by the total "on" time (t) in seconds:

$$Q = I_a * t \quad (2)$$

In the case of electroporation, the total charge delivered is given as,

$$Q = I_p * \tau * n \quad (3)$$

Where, I_p is the current passing through the skin during the pulse as measured by the oscilloscope, τ is the pulse length (converted to seconds), and *n* is the number of pulses applied.

RESULTS AND DISCUSSION

There was no measurable permeation of fentanyl under passive conditions, i.e., when no iontophoresis or electroporation was used (Fig. 1). However, as seen in Fig. 1, there was a significant flux when iontophoresis was applied ($p < 0.005$). The flux decreased once the current was turned off after 5 h. This demonstrates the reversibility of enhanced delivery and the feasibility of delivery modulation by changing the electrical current parameters. The iontophoretic flux is about 80 μg/cm^2-h within 3 h of iontophoresis. The marketed Fentanyl (Duragesic®) patch has a transdermal flux of 2.5 μg/cm^2-h. This shows that an iontophoretic patch can deliver drug over 30 times faster compared to the marketed patch, which contains ethanol, a skin permeation enhancer. Therefore, iontophoresis can provide quick onset of action and deliver therapeutic dose quickly for treatment of acute pain while the currently marketed patch is more suitable for slower onset and consistent delivery for treatment of chronic pain. The

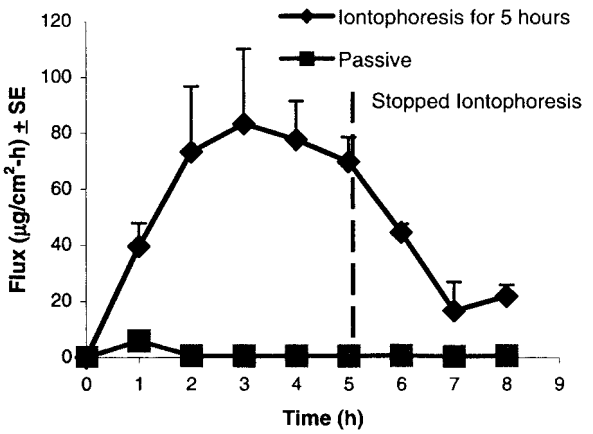

Fig. 1. Flux of fentanyl across human epidermis with passive delivery or with 5 h of iontophoresis.

iontophoretic patch can also provide drug delivery modulation to change flux as needed.

Figure 2 shows the effect of electroporation at time zero. If electroporation induced, (permeabilization) pores close in seconds; then pulses should be given periodically. However, if the pores are long lived, then all pulses can be given at the beginning. In a previous study, we have shown that the manner of pulsing did not have any significant effect on permeation (21). In this study, pulses were applied at the beginning at a rate of 1 pulse per minute. A very significant flux, much higher than that achieved by iontophoresis, is achieved and the lag time of delivery is also significantly less than that achieved by iontophoresis ($p < 0.05$). Electroporation provided a flux that was three times higher than that achieved by iontophoresis and close to 270 times greater compared to passive delivery. Thus, electroporation may allow for an even quicker onset of analgesia by rapid delivery of fentanyl within minutes compared to an hour to couple of hours with iontophoresis and several hours to a day with passive delivery. However, it should be noted that using this high intensity electroporation protocol in an *in vivo* setting would require careful development of electrodes and pulsing protocols to avoid intolerable electrical sensations and develop a patient compliant, cost-effective delivery device. The intensity required for *in vivo* delivery will, however, be less than the *in vitro* protocol as the electrodes will be in direct contact with the skin and an equivalent transdermal voltage can be reached at lower settings. The field strength is the voltage across the electrodes divided by the electrode spacing, though a correction for the voltage drop at the electrode interface may be required (23). It is expected that the voltage consumed by the medium will be minimized for *in vivo* setting, due to the close contact between skin and electrodes. Alterations in skin induced by high voltage pulses have been shown to be equivalent to those induced by iontophoresis of 0.5 mA/cm^2 for 1 h, which is considered to be safe. By concentrating the electrical field in the stratum corneum, side effects of sensations can be minimized as the nerves are located lower in the skin. High voltage pulses have been used in clinical studies with electrochemotherapy, though the high voltages used cause instantaneous painless contractions of the underlying muscles during pulse delivery. Electrode geometry affects the distribution of electric field within the tissue and improved design microfabricated electrode arrays are under development for clinical use to minimize sensations (24,25). The first report on clinical investigation of skin anesthesia using noninvasive skin electroporation or iontophoresis was published recently (26). The electrical sensation induced by twelve pulses (80 V, 10 msec) with surface-type electrodes was mild and well tolerable. As seen in Figure 2, the flux is found to recover quickly following electroporation pulsing. However, if electroporation is followed by iontophoresis, the flux is found to recover partly and then maintain during the period of iontophoresis. The flux then is gradually decreased following termination of iontophoresis. The flux obtained using a combination of iontophoresis and electroporation was not significantly different than that achieved by electroporation alone at 15 m but was greater at 5 h ($p < 0.05$). The slope of the cumulative amount of fentanyl delivered by these two means was significantly different. Figure 3 and Fig. 4 show that delivery can be modulated and controlled by repeating the electroporation protocol at different time intervals. As can be seen from Fig. 4, the flux recovered within each 1.5-h window and increased to a similar peak or slightly higher level when skin was pulsed repetitively. This shows at least a partial reversibility of the permeability of the skin after each pulsing and thus the feasibility of a controlled modulated delivery device can be investigated.

Figure 5 shows the transdermal voltages at each of the pulses for the experimental protocol of Fig. 3. It demonstrated that an applied voltage of 500 V results in an actual transdermal voltage around 40–70 V. The transdermal voltage gradually decreases slightly a little on each subsequent pulse, perhaps because the skin has been permeabilized by the previous voltage pulses. The voltages also seem to be lower on the subsequent application of pulses at 2.5 h. However, pulsing at 0, 1.5, 3.0, and 4.5 h did not show a definitive trend (data not shown). Though the flux after each pulsing protocol in Figs. 3 and 4 recovered almost completely within a period of minutes to hours, an increase in flux was observed for each of the pulsing protocol. There was an increase in current in both the 2-cycle (0.72 to 0.78 A) and 4-cycle (0.77 to 0.85 A) electroporation. This increase in current passing through the skin was observed to be higher in each consecutive pulsing protocol and this in turn may be due to a significant drop in skin resistance and a small drop in transdermal voltage that was observed after each pulsing protocol. Furthermore, any drug remaining in the skin may be contributing to a higher flux upon subsequent pulsing. The skin impedance

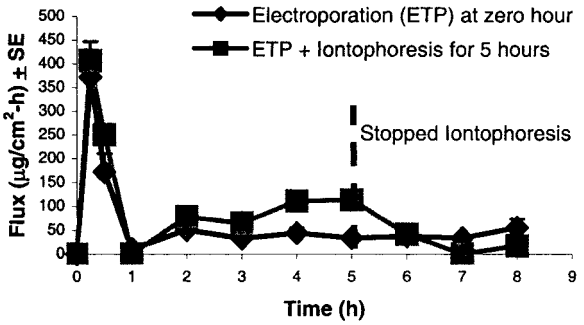

Fig. 2. Flux of fentanyl across human epidermis with electroporation consisting of 15 pulses (500 V, 200 msec), applied once every minute at time zero.

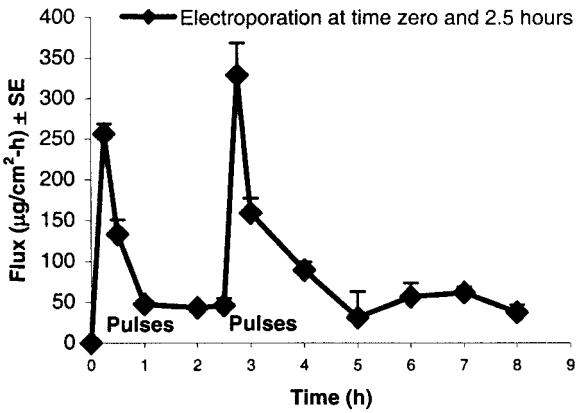

Fig. 3. Flux of fentanyl across human epidermis with electroporation consisting of 15 pulses (500 V, 200 msec), applied once every minute at time zero and 2.5 h.

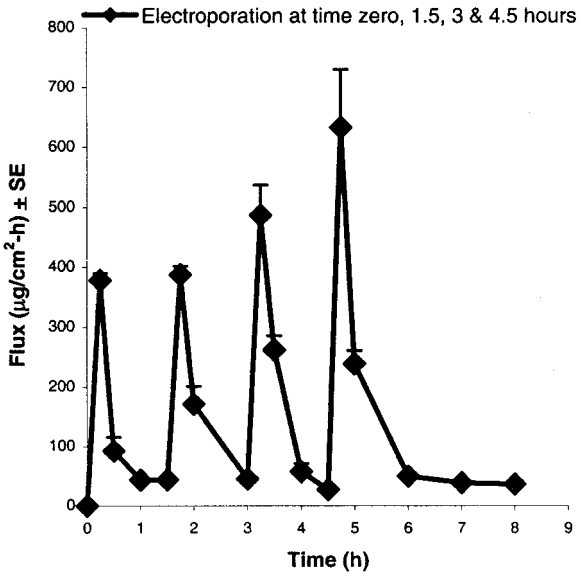

Fig. 4. Flux of fentanyl across human epidermis with electroporation consisting of 15 pulses (500 V, 200 msec), applied one every minute at time zero, 1.5, 3.0, and 4.5 h.

and its recovery were also monitored for the 4-cycle electroporation protocol (Fig. 6). The impedance was found to drop exponentially upon applying the first set of pulses and did not recover during the duration of the experiment. However, as seen before, the delivery of fentanyl did recover after each pulsing cycle. The current passing through the skin during these electroporation pulses was about 0.74–0.82 Amperes. The charge delivered during electroporation was 1.66, 3.33, or 5.83 Coulombs for one (applied at time zero only), two (applied at 0 and 2.5 h), and four (applied at 0, 1.5, 3.0, and 4.5 h) cycles of electroporation, respectively. The charge delivered across the skin during the 5-h iontophoresis period was 5.76 Coulombs. Thus, the charge delivered by iontophoresis was equivalent to the charge delivered by four-cycles of electroporation. A comparison of the corresponding cumulative amounts of fentanyl delivered by electroporation or iontophoresis (for the same charge delivered) is shown in Fig. 7. The difference is statistically significant ($p < 0.05$) at all time points. Iontophoresis for 5 h has delivered about the same amount of fentanyl compared to 1-cycle of electroporation. A

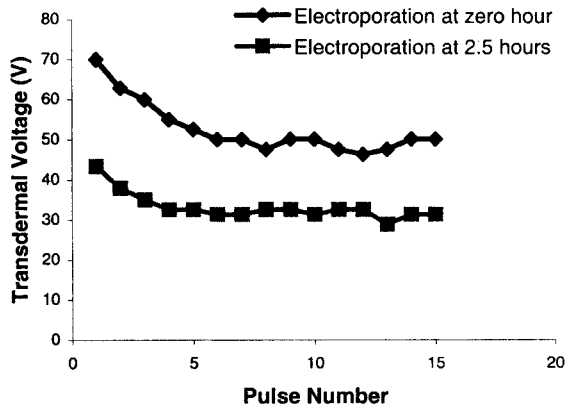

Fig. 5. Transdermal voltage for each of the 15-electroporation pulses (500 V, 200 msec), applied once every minute at time zero and 2.5 h.

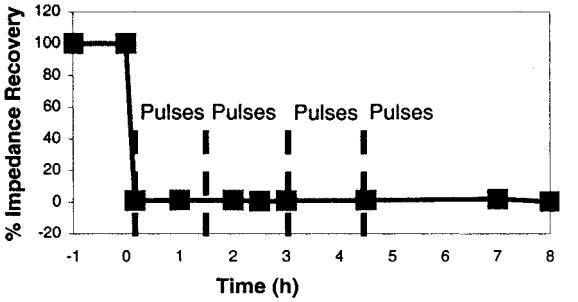

Fig. 6. Impedance of the human epidermis following electroporation consisting of 15 pulses (500 V, 200 msec), applied once every minute at time zero, 1.5, 3.0, and 4.5 h.

combination of iontophoresis and electroporation delivered the same amount of drug as 2-cycles of electroporation. The cumulative profile of fentanyl transport across human epidermis indicates that a rapid and sustained transdermal delivery can be programmed by using the appropriate parameters of electroporation or iontophoresis or the combination. However, electroporation appears more rapid and effective in delivering fentanyl compared to iontophoresis. For example, it takes 15 min of electroporation mode to reach a cumulative fentanyl delivery of 100 $\mu g/cm^2$, while it takes about 2 h of iontophoresis to achieve the same delivery. Electroporation results in about 100% increase in fentanyl delivery compared with iontophoresis at any given time-points (Fig. 7).

There may be two possible mechanisms producing enhancement in transdermal fentanyl delivery via electroporation. First, direct electrical repulsion similar to that seen in iontophoresis plays an important role for the rapid onset of fentanyl flux during the pulsing (Fig. 2) since fentanyl is positively charged in the donor chamber. Electroosmosis may also be a contributing factor during the time the pulse is applied, though its contribution may be minimal due to the short pulse lengths. Secondly, electroporation enhanced passive diffusion may contribute to a rise of fentanyl flux since the permeability of the epidermis is increased dramatically due to electroporation-induced transient alteration of skin structure. The application of iontophoresis and electroporation resulted in a

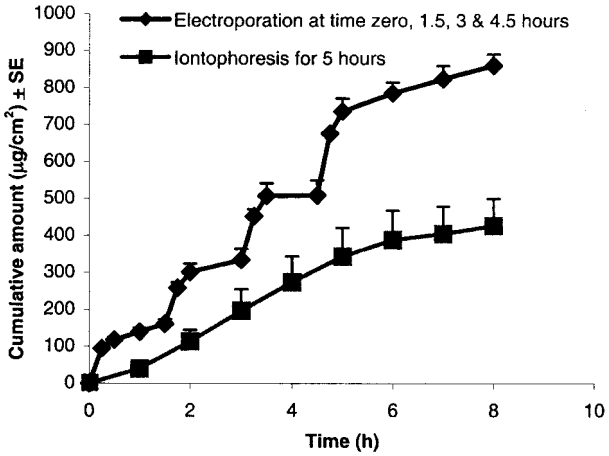

Fig. 7. Cumulative amount of fentanyl delivered through human epidermis with 5 hours of iontophoresis (5.76 Coulombs) or with electroporation consisting of 15 pulses (500 V, 200 msec), applied once every minute at time zero, 1.5, 3.0, and 4.5 hours (5.83 Coulombs).

higher fentanyl flux than electroporation or iontophoresis alone (Figs. 1 and 2). This is likely since iontophoretic-driving force provides more transfer of drug molecules through not only the existing pathways of the skin but also electroporation-induced pores in the skin (before they close completely). To our knowledge, this is a first demonstration of the feasibility of transdermal delivery of fentanyl with rapid onset and modulation profile (Fig. 4 and Fig. 7). Thus, electrically assisted transdermal delivery systems could be developed and provide therapeutic levels of serum fentanyl for treating either acute or chronic pain.

In conclusion, electrically assisted delivery can significantly improve the *in vitro* efficiency and shorten the lag time of transdermal transport of fentanyl compared to passive delivery. For the same charge delivered, the pulsing protocol was found to be more effective in this study as compared to iontophoresis. It is assumed in this study that the pulsing protocols induce skin electroporation. The modulation of electroporation or combination of iontophoresis and electroporation offers an advantage for potential clinical applications of improved delivery of fentanyl in analgesia and pain management. An on-demand patient controlled delivery may be achieved by appropriately programming the electrically assisted delivery systems.

ACKNOWLEDGMENTS

This work was funded by Genetronics, Inc. (San Diego, CA).

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