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Transdermal delivery of fentanyl: rapid onset of analgesia using skin electroporation

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Abstract

Skin electroporation has recently been shown to increase transdermal transport of small-size drugs as well as considerably larger molecules by up to 4 orders of magnitude in vitro. Nevertheless, no in vivo studies have proven that high-voltage pulses can induce therapeutic plasma levels of drug. The aim of the present report was precisely to study the potential of skin electroporation in transdermal delivery of fentanyl in vivo. Fentanyl was transdermally delivered to hairless rats using high-voltage pulsing. Following the administration, the pharmacokinetics and pharmacodynamics were assessed. Significant fentanyl plasma concentrations were rapidly achieved using skin electroporation. Immediately after the 5 min pulsing, fentanyl plasma levels reached one third of the maximal plasma concentration of ~30 ng/ml, the peak occurring 30 min after the electroporation. Deep analgesia and supraspinal effects were achieved, antinociception lasting for an hour. The magnitude of the effects was, however, dependent on the electrical parameters of the pulses. © 1998 Elsevier Science B.V.

Keywords: Skin electroporation; Fentanyl; Transdermal drug delivery; Iontophoresis; In vivo

1. Introduction

Recent reports have documented the value of non-invasive administration routes of potent opioids, including transdermal delivery [1–6], iontophoresis [7–9], oral transmucosal delivery [10,11], and nasal delivery [12]. These systems have been studied for their use in providing preoperative sedation and anxiolysis in pediatric patients [11], as well as analgesia for post-operative [2,5] and breakthrough cancer pain [5,6].

More specifically, passive transdermal delivery of fentanyl has been developed for the management of moderate to severe chronic pain [2–6]. After placement of a fentanyl transdermal system (Duragesic®), mean serum concentration increases during the first 14 h before reaching a plateau [3]. However, pain treatment may require rapid and pulsed administration of analgesic drugs. Opioid iontophoresis reduces the lag time to approximately half an hour and allows modulation of the rate of drug release by controlling the intensity and duration of current application [7–9]. Oral transmucosal fentanyl citrate (Fentanyl Oralet®) is a non-invasive dosage form of fentanyl used to provide children and adults with sedation, anxiolysis and analgesia [10,11]. The action is rapid (the peak in plasma concentration

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occurred after 20 min) and short (2 h) [10]. Intranasal sufentanil was studied for pre-operative sedation. The sedation was rapid in onset (10 min) but of limited duration (1 h) [12].

Several in vitro studies have demonstrated the usefulness of high-voltage electric field pulses in transdermal drug delivery: transdermal transport of small-size drugs as well as considerably larger molecules increases by up to four orders of magnitude, onset times for transport and lag times to reach steady-state fluxes decrease to minutes [13–16]. Experimental [13–16] and theoretical [17,18] evidence suggests these flux increases are caused by transient changes in skin microstructure by a mechanism involving electroporation of stratum corneum lipid bilayers. Electroporation involves the creation of transient aqueous pathways in lipid bilayers by the application of a short high-voltage electric field pulse.

Our previous reports evaluated the potential of electroporation in fentanyl transdermal delivery in vitro with hairless rat skin [19–21]. The influence of the electrical factors and the mechanistic aspects involved in drug transport were systematically investigated. Fentanyl transdermal permeation was strongly promoted by electric pulses as compared to passive diffusion through untreated skin. Moreover, the control of the quantity of drug transported through the skin was achieved by controlling the voltage, duration, number of pulses and drug concentration [19,20]. The mechanisms of drug transport involved electrophoresis and diffusion through skin highly permeabilized by electroporation [21].

Although fairly extensive work on skin electroporation has already been performed in vitro, high-voltage pulses have never been demonstrated to provide transdermal delivery of drug at therapeutic rates in vivo. We therefore investigated the potential of electroporation in fentanyl transdermal delivery in vivo. In vivo assays were performed in hairless rats to assess the pharmacokinetics and pharmacodynamics of fentanyl delivered by high-voltage pulses, and in comparison by iontophoresis or subcutaneous injection. The pharmacological evaluation included the measurement of the analgesic effect along with an evaluation of the supraspinal side effects on pinna and cornea reflexes and on skeletal muscle tone [7,22–24].

2. Materials and methods

2.1. Animals and chemicals

Hairless male rats, 2 months old, weighing 220–310 g were housed in standard cages at room temperature on a 12-h light and 12-h dark cycle (Iops mutant from Iffa Credo, France). Standard laboratory food (A04, UAR-France) and water were given ad libitum. Fentanyl citrate was purchased from Janssen Pharmaceutica. Salts for buffer preparation were analytical grade (Union Chimique Belge, Drogenbos, Belgium).

2.2. Treatments of the rats

A fold of abdominal skin of the rat was clamped into a clip. The clip was composed of two compartments each containing a polyurethane hydrophilic foam of 1 cm thickness and 1×3 cm^2 surface. A platinum electrode of 0.5×2 cm^2 (99.99% purity, Aldrich Chemie, Belgium) was at the outer surface of each foam. Cathode and anode sides were soaked with an acidic solution of fentanyl (400 μg/ml in citrate buffer 0.01 M at pH 5), or with the drug-free buffer. The electrodes were connected either to the electroporation device Easyject Plus (Equibio Ltd., Kent, UK) for skin electroporation, or to a constant current source for iontophoresis (custom-built device). Easyject Plus delivers exponential-decay electric field pulses of variable voltage and duration. The pulse duration is defined as the length of time between the beginning of the pulse (maximum voltage) and the time when the voltage reaches 37% of its maximal value. During a pulse, electrical behavior was measured with an oscilloscope (model 54602B, Hewlett-Packard). The voltage applied across the electrodes of the clip was measured directly. The corresponding peak current was calculated using Ohm's law by measuring the voltage across a sampling resistor (5 Ω) in series with the clip. Pulses of 100 V — 500 ms, 250 V — 200 ms or 500 V — 1.3 ms were used, reported voltages being voltages applied across the electrodes. The total electric charge transferred by a pulsing protocol was calculated by multiplying the peak current, pulse duration and number; the total charge transferred during an

iontophoresis protocol was calculated by multiplying current times the duration of application [16].

Subcutaneous injections were carried out with freshly prepared solutions of fentanyl citrate (8 μg/ml) as an aqueous solution in NaCl 0.9%.

For pharmacodynamic evaluation, skin electroporation or iontophoresis were applied to non-anesthetized rats kept motionless in a restraining cage. High-voltage pulsing caused sensation and slight muscle twitches in the rats [25]. Therefore, at the beginning of the pharmacokinetic studies, rats were briefly anesthetized by ether breathing for the 5 min of pulse application.

2.3. Pharmacodynamic study

2.3.1. Analgesia assay

Rats were tested for nociceptive response by using the tail-withdrawal reaction (TWR) method [22,23]. The rat was placed in a cylindrical rat holder with its tail hanging freely outside the cage. The distal 5 cm of the tail was immersed in a warm water bath at 55 ± 0.5°C and the time for tail withdrawal was measured to the nearest 0.01 s. To minimize tissue damage at repeated testing, a cut-off time of 10 s was used. A TWR latency >5.00 s and thus, also ≥10.00 s never occurred in untreated control rats and were adopted as criteria of mild and deep analgesia, respectively [22,23].

2.3.2. Other in vivo effects

The blockade of the cornea and pinna reflexes and muscular tone were scored as indices of supraspinal pharmacologic activity of the opioid [22–24]. Blockade of the cornea and pinna reflexes are characteristic effects of opioid analgesics at the level of the tenth and fifth cranial nerves, whereas rigidity probably originates in the striatum and substantia nigra. Supraspinal effects require approximately two-fold higher doses of fentanyl than antinociception [23]. The pinna reflex consisted of a characteristic head twitch induced by a gentle mechanical stimulation of the inner ear with a blunt metal rod (diameter 1.5 mm). The response of the animal was scored from 0 (normal reflex) to 3 (absence of any motor response). Scores 1 and 2 indicate that the reflex was slightly or markedly attenuated, respectively. The cornea reflex was examined by stimulation of the eye by a gentle mechanical stimulation with the blunt metal rod and was also scored from 0 (normal reflex) to 3 (absence of any motor response). Muscular tone was determined by hand and visually. The scores given for overall skeletal muscle tone ranged from 0 (normal tone) to 3 (lead pipe rigidity); scores 1 and 2 represent weakly and moderately increased tone, respectively. In untreated control animals, a score >1 never occurred for the pinna and cornea reflexes and for muscle tone. A score of 3 was used as the criterion of significant blockade of the pinna and cornea reflexes and of significant induction of muscle rigidity [22,23].

2.3.3. Experiments

Skin electroporation of fentanyl was carried out using different pulse protocols: 15 pulses of 100 V — 500 ms (group of five rats), or 15 pulses of 250 V — 200 ms (group of four rats), or 60 pulses of 500 V — 1.3 ms (group of four rats). In order to obtain the shortest treatment, pulses were applied at the shortest possible interval; due to the electroporation device limitation, the pulse spacing was then 15 s. A fourth group (five rats) underwent a 0.5 mA/cm^2 — 5 min iontophoresis of fentanyl and a fifth (four rats) received 4 μg per 100 g body weight subcutaneous fentanyl. Finally, three groups were controls: (1) the rat was maintained in the restraining cage during 5 min with the clip keeping a skin-fold and the foams being soaked with the drug-free citrate buffer (control, four rats), (2) same as (1) but with the application of the strongest pulses i.e. 15 pulses of 250 V — 200 ms in order to measure a possible analgesic effect due to pulsing alone (control pulsing, four rats), (3) same as (1) but instead the foam being soaked with the drug solution and for a duration of 15 min (duration of the longest skin electroporation treatment) in order to evaluate possible fentanyl effects due to passive diffusion (control diffusion, four rats).

Measurements of TWR latency and supraspinal side effects were taken before, immediately after, and then every quarter of an hour until 6 h after treatment.

2.3.4. Data analysis

At each experimental condition, the test results of the four or five rats were collected. Data are

expressed as median (minimum–maximum) (Figs. 1–4).

For further analysis, TWR latencies were converted to percentage of maximal possible effect (% MPE) according to the formula:

$$\%\,MPE = \frac{\text{postdrug latency} - \text{predrug latency}}{\text{cut} - \text{off time (10 s)} - \text{predrug latency}}.$$

The area under the effect curve (AUEC) from 0 to 2 h was calculated by accumulating % MPE measured at the discrete time intervals by using the trapezoidal rule and expressed as percentage of maximal possible AUEC i.e. AUEC for % MPE equals 100 from 0 to 2 h (Table 1).

Differences in measurements between experimental conditions were evaluated using the Mann-Whitney U-test ($P<0.05$).

2.4. Pharmacokinetic study

In another set of experiments, fentanyl plasma concentrations were measured after transdermal delivery of fentanyl by the 15 pulses of 100 V — 500 ms or by the 15 pulses of 250 V — 200 ms. Plasma samples were taken on anticoagulant (ethylenediaminetetraacetate; Sigma Chemical Co., St. Louis, MO) by tail incision immediately (within 1 min) after the electroporation treatment (time 5 min after the electroporation started), and at 15 min, 35 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, and 6 h after the beginning of the electroporation treatment. Plasma samples were stored at -20°C until analyzed. Fentanyl plasma concentrations were determined without extraction by radioimmunoassay (RIA) (Janssen Biotech, Beerse, Belgium) [7]. The limit of detection and accuracy of the assay was 0.1 ng/ml and 6%, respectively. Plasma concentrations were expressed as mean±S.E.M. of 8–14 rats (Fig. 5, Table 3). The area under the curve (AUC) was measured by the trapezoidal rule (Table 3). Fentanyl kinetics were compared by a two-way analysis of variance (ANOVA, $P<0.05$), peak fentanyl plasma concentrations (C_{max}), times for peak plasma concentrations (t_{max}) and AUC with the Student's t-test ($P<0.05$).

2.5. Skin tolerance

Skin erythema was evaluated visually immediately after and at 6 and 24 h after the electrical treatments of the pharmacodynamic study (Table 4). Score 0 was no redness, score 1 was perceptible redness, score 2 redness, score 3 high redness, and score 4 high redness with extension.

3. Results

3.1. Pharmacodynamic study

Fentanyl was delivered by skin electroporation using 15 pulses of 100 V — 500 ms, or 15 pulses of 250 V — 200 ms, or 60 pulses of 500 V — 1.3 ms. Because significant voltage drop occurred within solution soaking the foam, transdermal voltages were only a fraction of voltages applied across the electrodes, this fraction depending on the relative resistance of the solution and skin. Skin resistance decreased by up to three orders of magnitude during a pulse, the greater the voltage the higher the resistance drop [16]. Application of 100, 250 and 500 V across the electrodes then corresponded to 35, 45 and 30 V applied across the skin, 300, 150 and 30 Ω skin resistance and to 0.04, 0.1 and 0.3 A/cm^2 peak current densities, respectively.

While the 100 and 250 V protocols are typical of our previous in vitro studies [14,19–21], the 60 500 V pulses are typical of protocols used by others [13,16,26,27]. Because during pulsing, electrophoresis has been shown to be the main mechanism of molecular transport, fentanyl percutaneous penetration by high-voltage pulses is expected to depend on the total charge transferred by the pulses [13,14,21]. The 15 pulses of 100 V — 500 ms and the 15 pulses of 250 V — 200 ms transferred a similar total charge of 0.9 C and 1.1 C, respectively; and the 60 pulses of 500 V — 1.3 ms protocol transferred 0.06 C. A study of skin tolerance using the same electroporation protocols has been conducted, allowing to evaluate the correlation between transdermal drug transport and skin tolerance [28].

No increase in tail-withdrawal reaction (TWR) latency was observed in the 'control', 'control pulsing' and 'control diffusion' rats nor was there an effect on the pinna and cornea reflexes and on muscle tone (U-test $P>0.05$, Figs. 1–4b, Tables 1 and 2). In contrast, the 100 V/fentanyl treatment

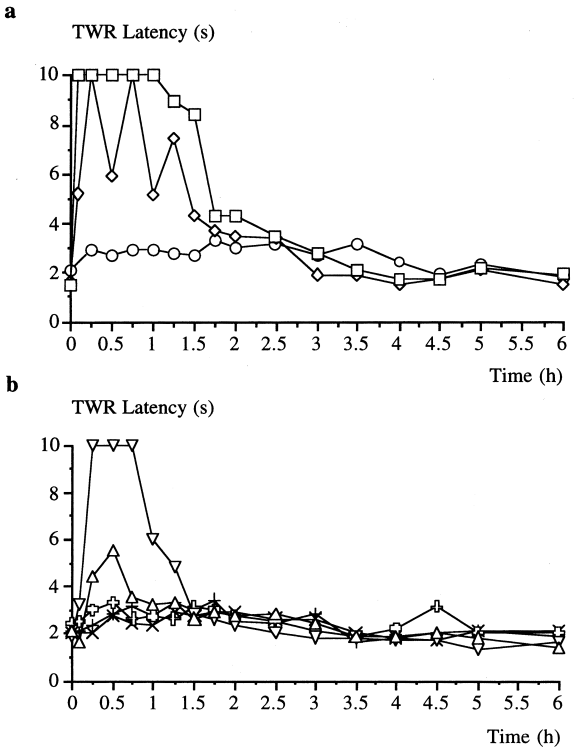

Fig. 1. Tail-withdrawal reaction (TWR) latency in the rat vs. time. (a) (◇) After 15 pulses of 100 V — 500 ms, (□) 15 pulses of 250 V — 200 ms, or (○) 60 pulses of 500 V — 1.3 ms. Pulse spacing was 15 s, then pulse application lasted from time 0 to 5 or to 15 min. Foams at the cathode and anode were soaked with an acidic solution of fentanyl (400 μg/ml in citrate buffer 0.01 M at pH 5). (b) (△) After an iontophoresis of 0.5 mA/cm^2 lasting from time 0 to 5 min, foams at the cathode and anode were soaked with an acidic solution of fentanyl (400 μg/ml in citrate buffer 0.01 M at pH 5). (▽) After a subcutaneous injection of fentanyl (4 μg/100 g) was performed at time 0. (✣) After holding the rat in the restraining cage from time 0 to 5 min, foams being soaked with the drug-free citrate buffer (control). (+) Same as control but with the application of the strongest pulses i.e. 15 pulses of 250 V — 200 ms (control pulsing). (X) Same as control but instead foams being soaked with the drug solution and from time 0 to 15 min (control diffusion). Results are presented as median of four or five values.

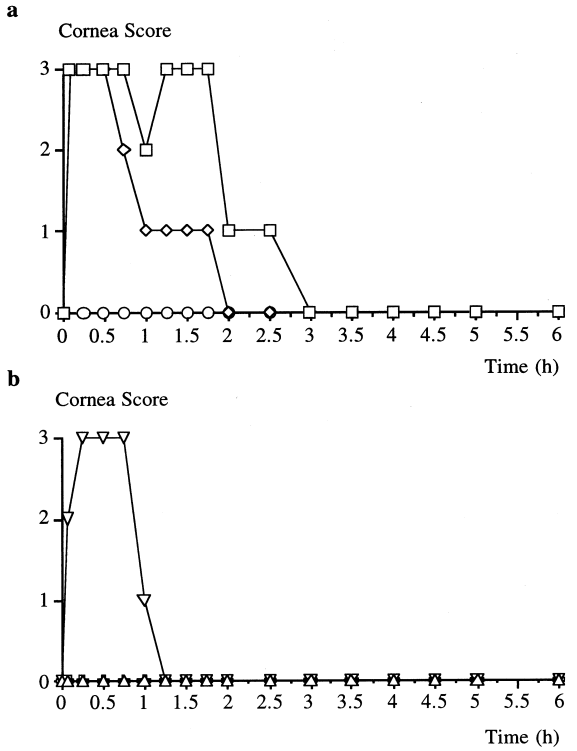

Fig. 2. Pinna reflex vs. time after the same treatments as in Fig. 1. None of the rats of the control groups had an effect on the pinna reflex then, only the data from the group control are shown (✣).

resulted already at the end of its application in an increased TWR latency (time 5 min, *U*-test $P<0.05$, Fig. 1a). The antinociceptive effect remained high up to 1.25 h after the beginning of the treatment, afterwards it declined to the control level (time 1.5 h, *U*-test $P>0.05$, Fig. 1a). The 250 V/fentanyl treatment did not result in a faster or stronger analgesic effect than the 100 V/fentanyl treatment except that the TWR latency recovered to control level slightly later: at time 1.75 h instead of 1.5 h (1.75 h, *U*-test $P>0.05$, Fig. 1a). However globally, % AUEC were equivalent (*U*-test $P>0.05$ 100 V vs. 250 V, Table 1a), and the duration of mild (TWR latency >5.00 s) and deep (TWR latency ≥ 10.00 s) analgesia were not significantly different (*U*-test $P>0.05$ 100 V vs. 250 V, Table 1b). The 500 V/fentanyl treatment did not induce any increase of TWR latency (*U*-test $P>0.05$, Fig. 1a, Table 1).

A significant blockade of the pinna and cornea reflexes were measured for the 100 V and 250 V/fentanyl treatments (*U*-test $P<0.05$ vs. control, Fig. 2 and Fig. 3a, Table 2). Significant muscle rigidity was observed only with the 250 V pulses (Fig. 4a, Table 2). None of the 500 V/fentanyl-treated rats showed an alteration of the pinna or cornea reflexes, or of muscle tone (Fig. 2, Fig. 3 and Fig. 4a, Table 2).

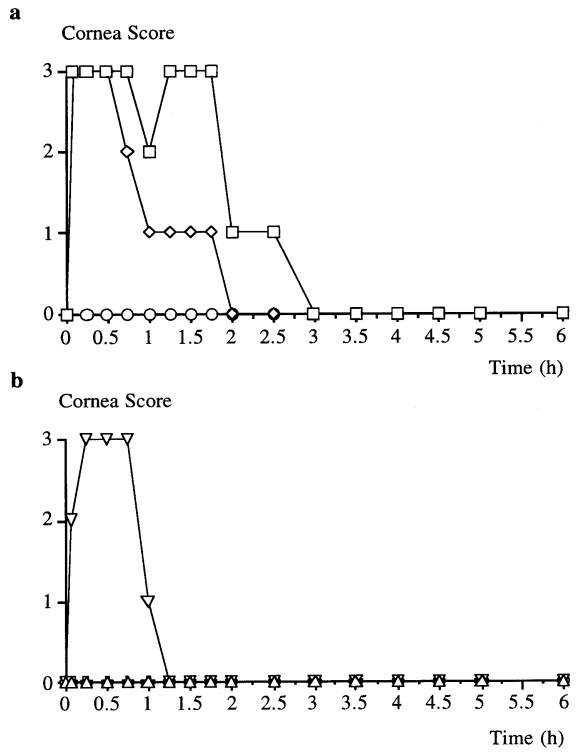

Fig. 3. Cornea reflex vs. time after the same treatments as in Fig. 1. None of the rats of the control groups had an effect on the cornea reflex then, only the data from the group control are shown (✣).

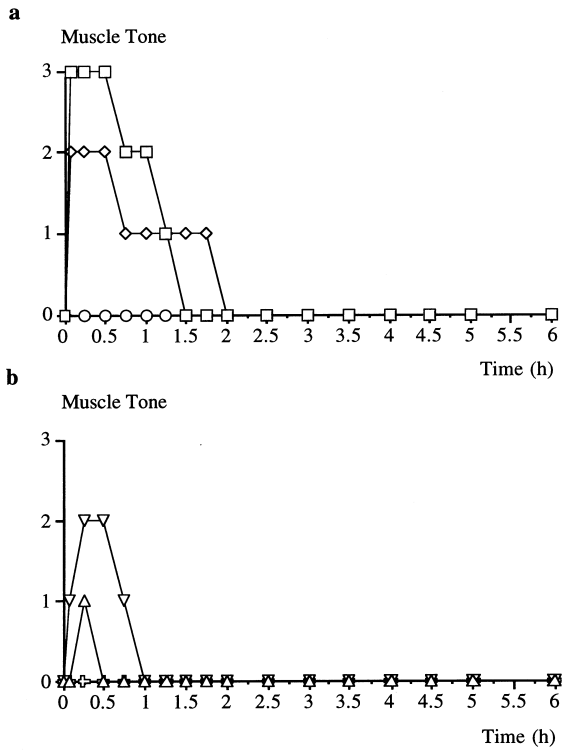

Fig. 4. Skeletal muscle tone vs. time after the same treatments as in Fig. 1. None of the rats of the control groups had an effect on muscle tone then, only the data from the group control are shown (✣).

For comparison, fentanyl was also delivered to the rat by iontophoresis. An iontophoresis of 0.5 mA/cm^2 was applied for 5 min. This intensity was chosen as being the maximum iontophoretic current clinically tolerated by patients, the 5-min duration because it provided the same duration of treatment as the 100 and 250 V electroporation treatments. This protocol transferred a total charge of 0.45 C. The TWR latency increased significantly only briefly at one discrete time point: 30 min after the beginning of the iontophoresis, 40% of the maximal possible analgesic effect was achieved (U-test $P<0.05$, Fig. 1b, Table 1a) however, overall the increase in % AUEC was not significant (U-test $P>0.05$, Table 1a). No significant increase was observed in the scores of the pinna and cornea reflexes nor on muscle rigidity (Fig. 2, Fig. 3 and Fig. 4b, Table 2).

In order to compare fentanyl transdermal delivery by skin electroporation and fentanyl delivery by the parenteral route, fentanyl was subcutaneously injected (4 μg/100 g). Five min after the injection, the TWR latency was not significantly increased ($P>0.05$, Fig. 1b). Fifteen min after the injection, it was increased to the maximum level of 10.00 s (U-test $P<0.05$, Fig. 1b, Table 1b). It remained high during 30 min and afterwards declined to the control level (time 1 h, U-test $P>0.05$, Fig. 1b). Significant blockade of the pinna and cornea reflexes lasted for about 25 min (Fig. 2 and Fig. 3b, Table 2). No significant induction of muscle rigidity was observed at this dose (Fig. 4b, Table 2).

3.2. Pharmacokinetic study

High fentanyl plasma levels were obtained after fentanyl delivery using the 15 pulses of 100 V —

Table 1
Comparison between the treatments in terms of (a) onset time of significant analgesia and corresponding % MPE and, AUC of % MPE vs. time (0–2 h) expressed as % of maximum possible AUC, and (b) duration of TWR latency >5.0 s or ≥10.0 s

Treatment	Onset		AUC % MPE 0–2 h
	Time (min)	% MPE	(%)
(a)			
15×(100 V — 500 ms)	5	47 (8–100)	51 (46–72)*
15×(250 V — 200 ms)	5	100 (36–100)	80 (50–95)*
60×(500 V — 1.3 ms)	No effect		9 (1–14)
Iontophoresis 0.5 mA/cm^2 — 5 min	30	40 (33–52)	18 (5–20)
Subcutaneous injection 4 μg/100 g	15	100 (84–100)	53 (32–71)
Controls	No effect		9 (−7–25)

Treatment	Duration (min) of TWR latency	
	>5.00 s	≥10.00 s
(b)		
15×(100 V — 500 ms)	70 (10–75)*	0 (0–55)
15×(250 V — 200 ms)	85 (15–115)*	70 (15–100)*
60×(500 V — 1.3 ms)	0 (0–0)	0 (0–0)
Iontophoresis 0.5 mA/cm^2 — 5 min	0 (0–15)	0 (0–0)
Subcutaneous injection 4 μg/100 g	50 (15–75)*	30 (0–55)*
Controls	0 (0–0)	0 (0–0)

Results are presented as median (min–max) of 4–5 values. *$P<0.05$ vs. control.

500 ms or 15 pulses of 250 V — 200 ms (Fig. 5). Directly after the 100 and 250 V/fentanyl treatment (time 5 min), plasma concentrations reached 12±2 and 14±4 ng/ml, respectively (*t*-test $P>0.05$ 100 V vs. 250 V, Fig. 5). Fentanyl plasma concentrations still further increased to reach a peak approximately half an hour after the treatment. Then, plasma concentrations slowly decreased. The fentanyl kinetics following the 250 V pulses were not statistically different from those following the 100 V pulses (ANOVA $P>0.05$, Fig. 5). Pharmacokinetic variables were equivalent as well (Table 3).

3.3. Skin tolerance

The electric treatments used in this study did not generally cause visible skin damages. A slight erythema was always observed immediately after

Table 2
Comparison between the treatments in terms of duration of significant blockade of the pinna or cornea reflexes, or of significant muscle rigidity

Treatment	Duration (min) of		
	significant blockade of		significant induction of
	pinna	cornea reflex	muscle rigidity
15×(100 V — 500 ms)	10 (0–40)*	25 (10–40)*	0 (0–10)
15×(250 V — 200 ms)	55 (25–115)*	70 (25–115)*	13 (10–15)*
60×(500 V — 1.3 ms)	0 (0–0)	0 (0–0)	0 (0–0)
Iontophoresis 0.5 mA/cm^2 — 5 min	0 (0–0)	0 (0–0)	0 (0–0)
Subcutaneous injection 4 μg/100 g	28 (15–55)*	23 (15–30)*	0 (0–0)
Controls	0 (0–0)	0 (0–0)	0 (0–0)

Results are presented as median (min–max) of 4–5 values. *$P<0.05$ vs. control.

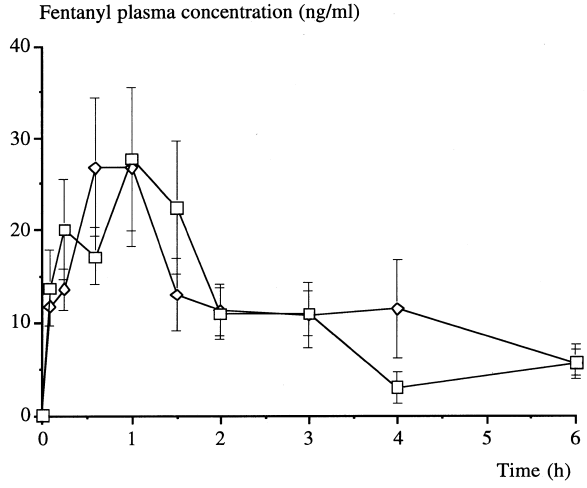

Fig. 5. Mean fentanyl plasma concentrations (±S.E.M.) vs. time after 15 pulses of 100 V — 500 ms (◇) or, 15 pulses of 250 V — 200 ms (□). Pulse spacing was 15 s then, pulse application lasted from time 0 to 5 min. Foams at the cathode and anode were soaked with an acidic solution of fentanyl (400 μg/ml in citrate buffer 0.01 M at pH 5) (n=8–14).

Table 3
Pharmacokinetic variables after fentanyl delivery by skin electroporation with 15 pulses of 100 V — 500 ms or 15 pulses of 250 V — 200 ms

Variable	100 V pulses	250 V pulses
C_{max} (ng/ml)	31±6	34±6
t_{max} (min)	35±6	48±9
$AUC_{0-6\ h}$ (ng.ml^{-1}.h)	66±14	70±16

Foams at the cathode and anode were soaked with an acidic solution of fentanyl (400 μg/ml in citrate buffer 0.01 M at pH 5). Data are mean±S.E.M., n=8–14.

treatment due to skin pinching by the clip (Table 4). While, the 250 V pulses seemed to slightly increase this redness, the 100 V and 500 V pulses and the iontophoresis did not seem to induce erythema by themselves (Table 4). No oedema, nor burn was observed [28].

4. Discussion

This study provides the first in vivo demonstration that skin electroporation may enhance the transdermal transport of a drug at therapeutic rates. Skin electroporation was shown to rapidly induce significant fentanyl plasma levels which were associated to strong analgesic effects: high-voltage pulses rapidly brought down the remarkable barrier function of the skin and fentanyl rapidly entered the systemic circulation. The onset of action was reduced to a few minutes, i.e. as fast as that obtained with a subcutaneous injection (Fig. 1, Table 1a).

Pharmacokinetic analysis and pharmacodynamic observations following pulsing were consistent since fentanyl plasma concentrations, and nociceptive and supraspinal scores showed a similar time profile (Figs. 1–5). The supraspinal side effects were always of shorter duration than the analgesia, in agreement with previous results (Table 1b and Table 2) [23]. Both plasma concentrations and pharmacodynamic effects of fentanyl increased rapidly. One third of the observed maximum fentanyl plasma concentration was already reached at the end of the 100 V or 250 V pulsing, the concentration peak

Table 4
Comparison between the treatments in terms of induced skin erythema: directly, 1, 6 and 24 h after treatment

Treatment	Skin redness			
	Directly	1 h	6 h	24 h
15×(100 V — 500 ms)	2 (2–3)	0 (0–1)	0 (0–1)	1 (0–2)
15×(250 V — 200 ms)	2 (1–3)	1 (0–2)	1 (0–2)	1 (0–2)
60×(500 V — 1.3 ms)	2 (0–3)	0 (0–0)	0 (0–1)	0 (0–0)
Iontophoresis 0.5 mA/cm^2 — 5 min	2 (0–3)	0 (0–0)	0 (0–0)	0 (0–1)
Control	1 (0–2)	0 (0–0)	0 (0–0)	0 (0–0)
Control diffusion	1 (0–2)	0 (0–0)	0 (0–0)	0 (0–0)
Control pulsing	2 (2–3)	1 (0–2)	1 (0–2)	2 (1–2)

Results are presented as median (min–max) of 4 or 5 values. 0 is no redness, 1 perceptible redness, 2 redness, 3 high redness and, 4 high redness with extension.

occurring around 30 min after pulsing. Although pulsing treatments lasted for only 5 min, fentanyl plasma concentrations increased for about 30 min suggesting that pulsing loaded the skin and/or underlying tissues with fentanyl which progressively diffused to the systemic circulation thereafter. In agreement with the pharmacokinetic data, immediately after pulse application, a respectively mild (100 V — 500 ms) and deep (250 V — 200 ms) analgesia were induced. The antinociceptive effect lasted for about an hour and then quickly disappeared.

Our previous studies performed in vitro with hairless rat skin demonstrated that (1) high-voltage pulses strongly enhanced transdermal transport of fentanyl, (2) the control of the quantity of drug delivered can be achieved by the choice of the voltage, duration and number of pulses [20]. The present in vivo study confirms these results: (1) skin electroporation of fentanyl induced significant fentanyl plasma levels associated to dramatic antinociceptive effects, (2) the 15 pulses of 250 V — 200 ms induced fentanyl pharmacodynamic effects slightly stronger than the 15 pulses of 100 V — 500 ms, while the 60 pulses of 500 V — 1.3 ms failed to induce analgesia. Because the 250 V pulses transferred a slightly larger total charge (1.1 C) than the 100 V pulses (0.9 C), the slightly stronger fentanyl in vivo effects are consistent [13,14,21]. In comparison, the 500 V pulses transferred a 17-fold smaller charge (0.06 C), and no fentanyl in vivo effects were observed indicating no or weak transdermal fluxes. Pulsing with 115–270 V transdermal voltage and 1.1 ms pulse duration at 1 pulse/min for 1 h was nevertheless reported to allow molecular flux through human epidermis in vitro to reach a steady-state within minutes [16,29]. Although fentanyl transport due to the short 500 V pulses was not pharmacologically relevant in our in vivo study, significant passage might occur with increasing the pulse number and rate, drug concentration and/or skin surface area [19].

As compared to the 100 or 250 V skin electroporation, the 0.5 mA/cm^2 — 5 min iontophoresis of fentanyl performed here had very brief and weak pharmacodynamic effects, and a longer onset of action (30 min). Although the maximum current density tolerated by patients, i.e. 0.5 mA/cm^2, was chosen, the time-averaged current (0.1 C/min) was two-fold lower than those obtained with the 100 and 250 V pulsing (0.2 C/min). However, longer iontophoretic application could strongly enhance fentanyl transport [7,9]. When compared to passive transdermal transport, both skin electroporation and iontophoresis are reported to enhance, expedite and control fentanyl delivery [7–9,20]. Nevertheless, skin electroporation could have the advantage of still shorter onset of action and shorter electrical treatment over iontophoresis (Table 1a). Moreover, enhanced iontophoretic delivery by the prior application of a high-voltage pulse has already been reported in vitro [15].

In man, analgesia and respiratory depression during surgery are associated with fentanyl plasma concentrations of 2–5 ng/ml. Post-operative analgesia is effectively obtained with plasma concentrations ranging from 0.3 and 0.7 ng/ml [30]. The plasma concentrations obtained in the present study were greater than those usually expected in human therapy. However, plasma concentrations in patients would be likely to be lower and stay elevated longer than those in the rats under similar experimental conditions because of different pharmacokinetic parameters: larger human vs. rat distribution volume ($V_d = 180$ vs. 1 l), longer human vs. rat distribution half-life ($t_{1/2\alpha} = 20$ vs. 8 min), longer human vs. rat elimination half-life ($t_{1/2\beta} = 230$ vs. 45 min) [30–32].

In human therapy, the main benefits of skin electroporation of fentanyl would appear both in chronic and acute pain management. In chronic pain management, skin electroporation could provide (1) a dramatic reduction in lag time as compared to classical patches allowing a more rapid control of pain [3], (2) if the patch is maintained, a continuous delivery through sustained electropermeabilized skin [21], (3) a modulation of the amount of drug released by controlling the voltage, duration and number of pulses [20], (4) a pulsed release of drug according to patient demand [29]. In acute pain management, skin electroporation of fentanyl could offer advantages over the other delivery methods. (1) Skin electroporation could eliminate the discomfort and the risk of infection associated with the injections, (2) when compared to oral delivery, skin electroporation avoids the first pass gastrointestinal and hepatic metabolism and expedites the onset of analgesia [10], (3) it could adequately replace oral

transmucosal fentanyl when oral intake is not tolerated (e.g. post-operative or cancer patients) [10,11].

Except for a mild erythema in some cases, the electric pulses used in this study did not cause visible skin damages (Table 4). This agrees with more comprehensive investigations of skin after high-voltage pulse exposure: skin structure and functionality are not strongly affected after electroporation [25,28,33,34]. However, pulse protocols and apparatus must be optimized and further in vivo studies are needed before issues of safety and irritation can be fully assessed.

5. Conclusion

The aim of the present report was to study the potential of skin electroporation in transdermal delivery of fentanyl in vivo in the rat. We showed that using high-voltage pulses, significant plasma levels of fentanyl in rats were rapidly reached. We also confirmed previous in vitro observations: the onset of analgesia of several hours by passive diffusion (classical patch) was expedited to a few minutes by high-voltage pulsing, the amount of drug delivered depended on the electric parameters of the pulses. When compared to iontophoresis, skin electroporation induced fentanyl effects more rapidly.

Many unresolved issues — electrodes design, optimization of high-voltage pulse protocols, safety considerations — must be addressed before transdermal drug delivery by electroporation may be clinically applied. However, an ability to increase transdermal transport by orders of magnitude with lag times of only minutes may present significant advantages in delivery of analgesics and other drugs.

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References

[1] J. Hadgraft, R.H. Guy (Eds.), Transdermal Drug Delivery in Developmental Issues and Research Initiatives, Marcel Dekker, 1989.
[2] D.J.R. Duthie, D.J. Rowbotham, R. Wyld, P.D. Henderson, W.S. Nimmo, Plasma fentanyl concentrations during transdermal delivery of fentanyl to surgical patients, Br. J. Anaesth. 60 (1988) 614–618.
[3] J.R. Varvel, S.L. Shafer, S.S. Hwang, P.A. Coen, D.R. Stanski, Absorption characteristics of transdermally administered fentanyl, Anethesiology 70 (1989) 928–934.
[4] S.D. Roy, G.L. Flynn, Transdermal delivery of narcotic analgesics: pH, anatomical and subject influences on cutaneous permeability of fentanyl and sufentanil, Pharm. Res. 7 (1990) 842–847.
[5] L.Y. Lee, J.R. Lopez, Transdermal fentanyl, Ann. Pharmacother. 26 (1992) 1393–1399.
[6] R.K. Potenoy, M.A. Southam, S.K. Gupta et al., Transdermal fentanyl for cancer pain, Anesthesiology 78 (1993) 36–43.
[7] S. Thysman, V. Préat, In vivo iontophoresis of fentanyl and sufentanil in rats: pharmacokinetics and acute antinociceptive effects, Anesthesiology 77 (1993) 61–66.
[8] S. Thysman, C. Tasset, V. Préat, Transdermal iontophoresis of fentanyl: delivery and mechanistic analysis, Int. J. Pharm. 101 (1994) 105–113.
[9] M.A. Ashburn, J. Streisand, J. Zhang, The iontophoresis of fentanyl citrate in humans, Anesthesiology 82 (1995) 1146–1153.
[10] J.B. Streisand, J.R. Varvel, D.R. Stanski et al., Absorption and bioavailability of oral transmucosal fentanyl citrate, Anesthesiology 75 (1991) 223–229.
[11] N.L. Schelter, S. Weisman, M. Rosenblum, B. Bernstein, P.L. Conard, The use of oral transmucosal fentanyl citrate for painful procedures in children, Pediatrics 95 (1995) 335–339.
[12] M. Vercauteren, E. Boeckx, G. Hanegreefs, H. Noorduin, G. Vanden Bussche, Intranasal sufentanil for pre-operative sedation, Anesthesia 43 (1988) 270–273.
[13] M.R. Prausnitz, V.G. Bose, R. Langer, J.C. Weaver, Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery, Proc. Natl. Acad. Sci. USA 90 (1993) 10504–10508.
[14] R. Vanbever, N. Lecouturier, V. Préat, Transdermal delivery of metoprolol by electroporation, Pharm. Res. 11 (1994) 1657–1662.
[15] D.B. Bommannan, J. Tamada, L. Leung, R.O. Potts, Effect of electroporation on transdermal iontophoretic delivery of luteinizing hormone (LHRH) in vitro, Pharm. Res. 11 (1994) 1809–1814.

[16] U. Pliquett, J.C. Weaver, Electroporation of human skin: simultaneous measurement of changes in the transport of two fluorescent molecules and the passive electrical properties, Bioelectrochem. Bioenerg. 39 (1996) 1–12.
[17] Y.A. Chizmadzhev, V.G. Zarnytsin, J.C. Weaver, R.O. Potts, Mechanism of electroinduced ionic species transport through a multilamellar lipid system, Biophys. J. 68 (1995) 749–765.
[18] D.A. Edwards, M.R. Prausnitz, R. Langer, J.C. Weaver, Analysis of enhanced transdermal transport by skin electroporation, J. Control. Release 34 (1995) 211–221.
[19] V. Préat, R. Vanbever, Transdermal drug delivery by electroporation, in: K.R. Brain, V.J. James, K.A. Walters (Eds.), Prediction of Percutaneous Penetration, 4b, Cardiff, 1995, pp. 26–29.
[20] R. Vanbever, E. Le Boulengé, V. Préat, Transdermal delivery of fentanyl by electroporation I. Influence of electrical factors, Pharm. Res. 13 (1996) 559–565.
[21] R. Vanbever, N. De Morre, V. Préat, Transdermal delivery of fentanyl by electroporation II. Mechanisms involved in drug transport, Pharm. Res. 13 (1996) 1359–1365.
[22] T.F. Meert, H. Noorduin, H. Van Craenendonck, P. Vermote, F.P. Boersma, P.A.J. Janssen, Effects of adrenaline, an α_2-adrenoceptor agonist, the volume of injection, and the global pain state of the animal on the activity of epidural fentanyl, Acta Anaesth. Belg. 40 (1989) 247–261.
[23] T.F. Meert, M. De Kock, Potentiation of the analgesic properties of fentanyl-like opioids with α_2-adrenoceptor agonist in rats, Anesthesiology 81 (1994) 677–688.
[24] H. Buerkle, T.L. Yaksh, Continuous intrathecal administration of short-lasting μ opioids remifentanil and alfentanil in the rat, Anesthesiology 84 (1996) 926–935.
[25] M.R. Prausnitz, The effects of electric current applied to the skin: a review for transdermal drug delivery, Adv. Drug Deliv. Rev. 18 (1996) 395–425.
[26] T. Zewert, U. Pliquett, R. Langer, J.C. Weaver, Transdermal transport of DNA antisense oligonucleotides by electroporation, Biochem. Biophys. Acta 212 (1995) 286–292.
[27] M.R. Prausnitz, E.R. Edelman, J.A. Gimm, R. Langer, J.C. Weaver, Transdermal delivery of heparin by skin electroporation, Biotechnology 13 (1995) 1205–1209.
[28] R. Vanbever, D. Fouchard, A. Jadoul, N. De Morre, V. Préat, J.-P. Marty, In vivo non-invasive evaluation of hairless rat skin after high-voltage pulse exposure. Skin Pharmacol. (in press).
[29] M.R. Prausnitz, U. Pliquett, R. Langer, J.C. Weaver, Rapid temporal control of transdermal drug delivery by electroporation, Pharm. Res. 11 (1994) 1834–1837.
[30] A. Poklis, Fentanyl: a review for clinical and analytical toxicologists, J. Toxicol. Clin. Toxicol. 33 (1995) 439–447.
[31] C.C. Hug, M.R. Murphy, Tissue redistribution of fentanyl and termination of its effects in rats, Anesthesiology 55 (1981) 369–375.
[32] S. Björkman, D.R. Stanski, D. Verotta, H. Harashima, Comparative tissue concentration profiles of fentanyl and sufentanil in humans predicted from tissue/blood partition data obtained in rats, Anesthesiology 72 (1990) 865–873.
[33] J.E. Riviere, A.N. Monteiro-Riviere, R.A. Rogers, D. Bommannan, J.A. Tamada, R.O. Potts, Pulsatile transdermal delivery of LHRH using electroporation: drug delivery and skin toxicology, J. Control. Release 36 (1995) 229–233.
[34] R. Vanbever, D. Skrypczak, A. Jadoul, V. Préat, Comparison of skin electroperturbations induced by iontophoresis and electroporation, in: K.R. Brain, V.J. James, K.A. Walters (Eds.), Prediction of Percutaneous Penetration, 4b, Cardiff, 1995, pp. 243–246.