Transdermal Delivery of Fentanyl by Electroporation I. Influence of Electrical Factors

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Purpose. Electroporation, a method of reversibly permeabilizing lipid bilayers by the application of an electric pulse, has been shown to induce increased transdermal passage of molecules. The aim of the present report was to study *in vitro* with hairless rat skin the potential of electroporation for transdermal delivery of fentanyl.

Results. The application of electric pulses can strongly promote transdermal delivery of fentanyl compared to passive diffusion through untreated skin. We also point out that the choice of the waveform of the electric pulses is important: at the same applied energy, a few exponentially-decaying (ED) pulses increased fentanyl permeation more than a few square-wave pulses and to the same extent as the repeated application of higher voltage-shorter duration ED pulses. A factorial design showed that the voltage, duration, and number of ED pulses allowed control of the quantity of drug transported through the skin.

Conclusions. Skin electroporation could be a good way to improve the transdermal diffusion of fentanyl.

KEY WORDS: transdermal drug delivery; fentanyl; electroporation; iontophoresis.

INTRODUCTION

The excellent barrier properties of the stratum corneum (SC), the outermost layer of the skin, to transport water and solutes crucially assists the body's regulation of water and electrolyte loss, and provides its ability to ward off the transdermal invasion of foreign substances. The SC is composed of corneocytes embedded in lipid domains consisting of alternatively hydrophilic and lipophilic layers. The barrier to permeation was attributed by several authors not only to the interstitial lipid composition but also to their structure as ordered multilayers (1).

Different approaches have been investigated to enhance skin permeability and to extend the transdermal application field to new drugs. Iontophoresis is an electrical method that has received widespread attention: a relatively low transdermal voltage (between 0.1 and 5 V across the skin) is used to drive molecular transport (2). Recent experimental and theoretical work supports the view that iontophoresis primarily involves electrically driven transport through fixed pathways across the SC.

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For the past ten years a physical method called electroporation or electropermeabilization, using the application of high voltage external electric field pulses, is routinely used in cell biology and biotechnology to introduce large molecular weight compounds (e.g. plasmids) into the cells (3). It was observed that electric fields larger than a threshold lead membranes to become transiently permeable. Recently, high voltage electric field pulses have been shown to increase rapidly transdermal flux of molecules up to four orders of magnitude (4–10). The mechanism implied is believed to be electroporation of the lipid bilayers of the SC (4–5). However, the interest of electroporation to deliver transdermally drug at therapeutic level has not yet been demonstrated.

Fentanyl is an opioïd used for analgesia and anesthesia. It is a basic (pKa = 8.9) lipophilic ($K_{oct/water\ at\ pH\ 7.4}$ = 717) molecule of a molecular weight of 336 Da (11). Fentanyl is currently formulated as a sterile solution for intravenous injection in the induction or maintenance of anesthesia. A fentanyl transdermal system is presently available: after a lag time of approximately 14 h, it provides a continuous systemic delivery of the drug for the management of chronic pain (11). The interest of iontophoretic transport of fentanyl has already been demonstrated: iontophoresis reduced the lag time and allowed control of the delivery of the drug (12–13).

The potential of electroporation for transdermal delivery of fentanyl was investigated. The aim of the present report was to study the influence of the waveform and polarity of the pulse *in vitro* using hairless rat skin. A factorial design was performed to evaluate the effect of the voltage, duration and number of the pulses on the control of fentanyl transdermal permeation. Physico-chemical parameters, mechanisms implied in fentanyl transport by electroporation and *in vivo* studies will be discussed in other report.

MATERIALS AND METHODS

Chemicals and Animals

Fentanyl citrate was purchased from Janssen Pharmaceutica (Beerse, Belgium). [H^3]-fentanyl was a gift from Janssen Biotech (Olen, Belgium). The salts used to prepare the buffers (for analysis) were supplied by UCB (RPL, Leuven, Belgium). All solutions were prepared in ultrapure water (Sation 900, Vel, Leuven, Belgium).

Skin for *in vitro* experiments was obtained from 2–3-month-old male hairless rats (mutant rat lops hairless from Iffa Credo, Saint Germain les Arbreles, France).

In Vitro Model and Procedures

Skin electroporation or iontophoresis was performed *in vitro* in horizontal chambers made of two compartments separated by skin (Makrolon, Obra, Liege, Belgium) (5, 7–9). The freshly excised full thickness abdominal skin of hairless rats was removed and mounted between the two compartments with stratum corneum facing the donor solution. The surface area of the membrane was 3 cm^2. Platinum electrodes (1 × 1 cm) (platinum pure, Aldrich Chemie, Bornem, Belgium) were immersed in the solutions (unless otherwise noted, the anode in the donor solution and the cathode in the receiver solution as fentanyl is +1 charge

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at pH 5). The distances between the upper (donor) electrode and the skin, and the under receiver electrode and the skin were respectively 0.3 cm and 0.7 cm. The electrodes were connected to an electroporation device for skin electroporation or to a constant current source for iontophoresis.

For all studies, the receiver compartment (7.5 ml) was filled with phosphate buffer (0.024 M) at pH 7.4 made isotonic with glucose, continuously stirred magnetically and maintained at 37°C. The upper reservoir was filled with 1.5 ml of the donor solution: fentanyl (40 μg/ml) citrate and [H^3]-fentanyl (0.5 μCi/ml) were introduced in a citrate buffer (0.01 M unless otherwise noted) at pH 5. After skin electroporation or iontophoresis, samples (0.4 ml) were taken from the receiver compartment at regular intervals for 6 h and replaced with an equal volume of drug-free phosphate buffer. Each sample was placed into a separate scintillation vial and mixed with liquid scintillation cocktail (Ready-Safe, Beckman, Belgium). The radioactivity of the vials was measured by β counting (Wallac 1410, LKB, Pharmacia). The ratio of the cumulative quantities detected in the receiver compartment to the skin area was plotted in term of time. The results were expressed as means ± the standard error of the means (n = 3 to 11).

A study of fentanyl stability under high voltage pulses exposure was conducted with the donor solution. An HPLC procedure was set up to analyse the solution. A column of octadecylsilane (μBondapak C18; 15 cm × 3.9 mm; Millipore, Waters, Brussels, Belgium) was used. The mobile phase was water (adjusted to pH 3 with H_3PO_4): acetonitrile 30/70 (v/v). The flow rate was 1 ml/min, and 10 μl aliquots were injected. Codeine phosphate was used as the internal standard. The UV detection was performed at 210 nm. No degradation of fentanyl was detected after the exposure to high voltage.

Electroporation

The electroporation device used was an Easyject Plus® (Equibio, Seraing, Belgium) delivering exponentially-decaying (ED) capacitive discharge pulses. The pulse time (τ) 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 initial value. τ can vary from 30 μs to 1 s. Easyject Plus® can be programmed to generate either single pulses -in high or low voltage mode- or, twin pulses consisting in a first high voltage pulse, an interpulse delay (fixed in this study at 0.1 s or 1 s), and a second low voltage pulse. Low voltage mode was used for voltage below 250 V (5, 7).

The energy of the electrical pulses (E) applied to the solutions and skin was calculated from the equation $E = N \cdot \tau \cdot (V_i^2 - V_f^2)/2. R_{chamber}$, where N is the number of the electrical pulses, τ is the pulse duration, V_i is the initial applied voltage, V_f is the final applied voltage (8 V in the low voltage mode and 34 V in the high voltage mode) and $R_{chamber}$ is the resistance of the diffusion chamber during the pulse (7).

An electroporation device T 820® from BTX generating square-wave (SW) pulses was also used. The pulse duration can vary from 0 to 99 ms. The applied energy of the SW pulses (E) was estimated by the equation $E = N \cdot D \cdot V^2/R_{chamber}$ where N is the number of the pulses, D is the pulse duration, V is the applied voltage and $R_{chamber}$ is the resistance of the diffusion chamber during the pulse estimated equal to $R_{chamber}$ during an equi-voltage ED pulse (8).

Unless otherwise noted, the pulses were separated by 1 min. Transdermal voltages which were approximately evaluated by the ratio of the skin resistance to the total chamber resistance (5, 7) were approximately 30% of applied voltages. Voltages are expressed as applied values and not transdermal values.

Statistical Analysis

The ratios of cumulative quantities detected in the receiver compartment to the membrane area were compared by Student t-test ($p < 0.05$ or < 0.01). The kinetics of drug permeation were compared by a two way analysis of variance (Scheffé F-test, $p < 0.05$ or 0.01). The analysis of the factorial design was performed with the help of the Systat package on Macintosh microcomputer (Systat: Statistics, Version 5.2 Edition. Evanston, II: Systat, Inc., 1992, 724 pp).

RESULTS AND DISCUSSION

Electroporation vs Iontophoresis

Both iontophoresis and skin electroporation have been reported to enhance transdermal passage of drugs (2, 4–10, 12–13). In order to compare the enhancement of fentanyl permeation through the skin induced by these electrical methods, iontophoresis was performed with experimental conditions close to clinical conditions (0.17 mA/cm² for 1 h) while skin electroporation was performed by the application of five pulses of (150 V − 300 ms) (5, 7–9).

The kinetics of fentanyl permeation were promoted to the same extent following both electric applications compared to diffusion through untreated skin (Fig. 1, $p > 0.05$ F-test).

The enhancement of fentanyl permeation by high voltage pulse let guess that skin electroporation could be interesting to improve fentanyl delivery of the transdermal diffusion system and, as iontophoresis to decrease the lag time of fentanyl systemic appearance. This enhancement can not be explained by high voltage iontophoresis. If we considered that changes in skin structure did not occur during the pulses i.e. that the skin

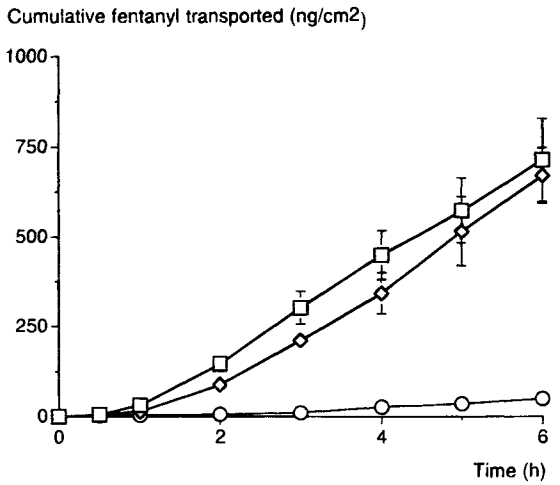

Fig. 1. Cumulative fentanyl transported (ng/cm²) vs time (h) after passive diffusion (○), iontophoresis (0.17 mA/cm² − 1 h) (◇) or electroporation (5 × (150 V − 300 ms)) (□). Fentanyl 40 μg/ml was introduced in a citrate buffer pH 5 (0.01 M).

is no more altered than during iontophoresis (same conductance estimated at 3 kΩ (9)), the five (150 V − 300 ms) pulses would transfer an amount of charge of 0.07 C, while the amount of charges transfered by the (0.17 mA/cm^2 − 1 h) iontophoresis is 1.8 C. So enhanced fentanyl permeation induced by skin electroporation can not be explained by electrophoresis alone suggesting that changes in skin structure must occur (4–5). Moreover, skin impedance dropped by two orders of magnitude during pulses application whereas it decreased only by one order of magnitude during iontophoresis (9).

Influence of Competitive Ions and Electrodes Polarity

The influence of the addition of competive ions in the donor solution and, of the polarity of the electrodes were evaluated. Fentanyl permeation following the application of five (100 V − 440 ms) did not decrease significantly when the buffer was ten times as concentrated (0.1 M in stead of 0.01 M) or when the cathode instead of the anode was placed in the donor compartment (Fig. 2, $p > 0.05$ t-test). These data suggest that passive diffusion of fentanyl through electropermeabilized skin is important in addition to the electrophoretic movement of the drug (5). As fentanyl is at a low concentration in comparison with the 0.01 M buffer, this behavior could be due to competition of the ions in the transport.

Influence of Pulse Waveform on Drug Permeation

Square Wave Pulses vs Exponentially-decaying Pulses

The efficiency of square-wave (SW) pulses and exponentially-decaying (ED) pulses to promote transdermal fentanyl permeation was compared at the same electric energy applied to the diffusion chamber. Fentanyl permation was followed after the application of five 100 V or 250 V SW pulses with a duration of 60 ms each and compared to the permeation following five 100 V or 250 V ED pulses with a duration of 125 ms (8). The 100 V SW and ED pulses correspond to an energy of approximately 9 J while, the 250 V SW and ED pulses correspond to an energy of approximately 67 J. The expression in term of energy was chosen instead of the expression in term of quantity of charges transported by the pulses because the transport mechanisms implied in drug transdermal permeation by electroporation were shown to be a contribution of both electrophoretic movement of the drug and increased passive diffusion through electropermeabilized skin (Fig. 2, 5, 7).

For both ED and SW pulses, fentanyl permeation increased through the skin as compared to diffusion through untreated skin. However, at both voltages the quantities of fentanyl transported were higher due to ED pulses than SW pulses (Fig. 3a, $p < 0.01$ F-test). In addition the five 100 V ED pulses caused the same fentanyl permeation as the five 250 V SW pulses (Fig. 3a, $p > 0.05$ F-test).

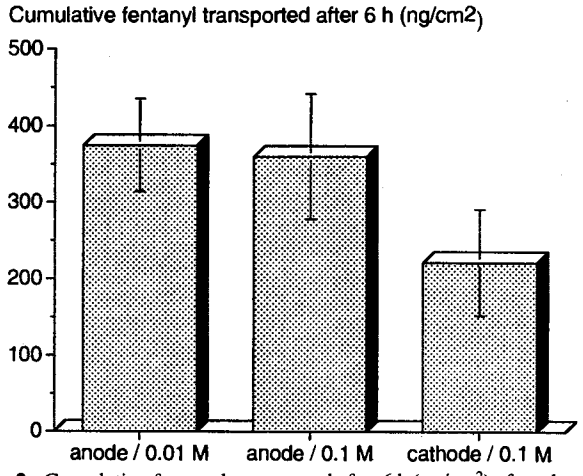

Fig. 2. Cumulative fentanyl transported after 6 h (ng/cm^2) after electroporation (5 × (100 V − 440 ms)) with the anode in the donor compartment and fentanyl (40 μg/ml) introduced in a citrate buffer pH 5 0.01 M or, 0.1 M or, with the cathode placed in the donor compartment and fentanyl (40 μg/ml) introduced in a citrate buffer pH 5 0.1 M.

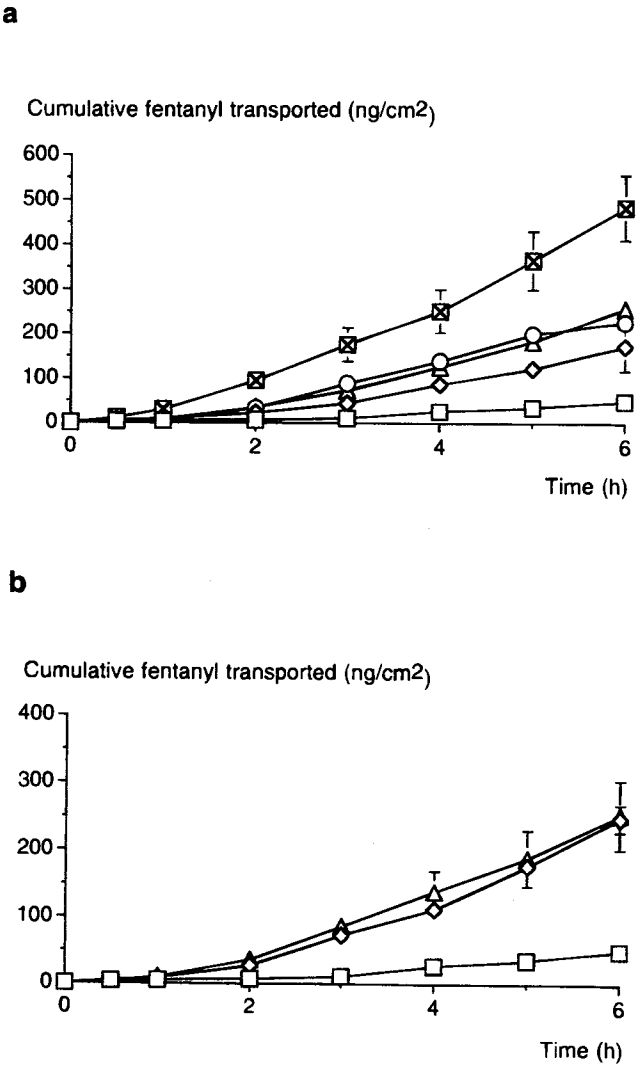

Fig. 3 a: Cumulative fentanyl transported (ng/cm^2) vs time after passive diffusion (□), 5 × (100 V − 60 ms) square wave (SW) pulses (◇), 5 × (250 V − 60 ms) SW pulses (○), 5 × (100 V − 125 ms) exponentially-decaying (ED) pulses (△), or 5 × (250 V − 125 ms) ED pulses (⊠). Fentanyl 40 μg/ml was introduced in a citrate buffer pH 5 (0.01 M). b: Cumulative fentanyl transported (ng/cm^2) vs time after passive diffusion (□), 60 × (300 V − 1.6 ms) ED pulses one applied every 10 s (◇), or after 5 × (100 V − 200 ms) ED pulses one applied every min (△). Fentanyl 40 μg/ml was introduced in a citrate buffer pH 5 (0.01 M).

Skin electroporation could be a two step phenomenon (7), as suggested by Rols and Teissié for cell electropermeabilization (14). The first step would consist of a voltage-dependent step of creation of permeated structures. The second step would involve the maintenance or expansion of these structures and/or electrophoretic movement of molecules, step depending on duration and number of pulses. Both ED and SW pulses permeabilized the skin efficiently but ED pulses could be more efficient than SW pulses to maintain or to expand these permeated structures and/or for electrophoretic movement of the drug.

Large Number of High Voltage Short Duration Pulses Vs Small Number of Low Voltage Long Duration Pulses

The effect on fentanyl transdermal transport of a large number of high voltage-short duration pulses (LN-HV) and of a small number of low voltage-long duration pulses (SN-LV) was compared at the same applied energy. The reason for this study was to compare transdermal permeation following the application of the pulses protocols used in our laboratory (SN-LV) (5, 7–9) and to those used by others (LN-HV) (4, 10). Sixty (300 V − 1.6 ms) with a pulse spacing of 10 s or five (100 V − 200 ms) pulses with a pulse spacing of 1 min were applied to the skin. These pulses provided an energy of approximately 24 J to the chamber and caused an equivalent increase in fentanyl permeation through the skin (Fig. 3b, $p > 0.05$, F-test). Increasing the pulse spacing between the LN-HV from 10 s to 1 min did not alter fentanyl kinetic (data not shown). It appears that the threshold of pulse duration to detect some increase in fentanyl permeation is low (lower than 1.6 ms) for high voltage.

Our previous report (7) showed a lower efficiency of LN-HV in comparison with SN-LV to promote metoprolol permeation. We also showed that metoprolol transport by electroporation implied mainly electrophoretic movement (5) while diffusion through electropermeabilized skin is important in fentanyl transport (Fig. 2). With this in mind, our interpretation is that the LN-HV could be efficient to permeabilize the skin but less efficient than the SN-LV to obtain an electrophoretic movement of the drug. Pliquett and Weaver (10) using short high voltage ED pulses (transdermal voltage around 200 V and pulse time 1.1 ms, one applied every minute for 1 h) to enhance transdermal permeation of molecules through human skin *in vitro,* have shown that the contribution of electrophoresis was important for calcein (−4 charge polar molecule) while diffusion dominated for sulforhodamine (−1 charge polar molecule). So the contribution of the different mechanisms implied in molecular transport by electroporation appear to depend on both electrical parameters of the pulses and physico-chemical parameters of the molecule and solutions (5, 7–8, 10). The SN-LV pulses could also be more adequate for clinical application since they allow a shorter treatment and a lower number of electrically induced muscles contractions (7–8).

Twin Pulses Vs Single Pulses

The application of five (24 V − 1.2 s) or five (300 V − 3 ms) pulses increased fentanyl permeation through the skin in an equivalent extent (Fig. 4, $p > 0.05$ F-test) suggesting again that a short duration pulse at high voltage caused an increase in fentanyl permeation.

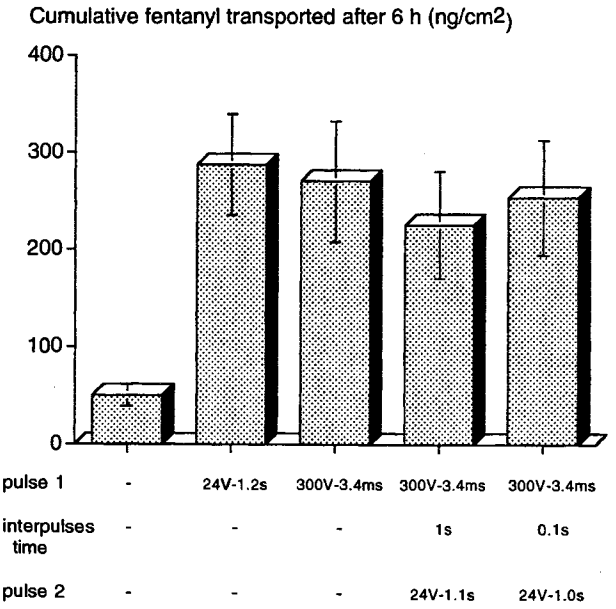

Fig. 4. Cumulative fentanyl transported after 6 h (ng/cm^2) after passive diffusion, 5 × (24 V − 1.2 s), 5 × (300 V − 3.4 ms), or 5 twin pulses: (300 V − 3.4 ms) followed after 1 or 0.1 s by (24 V − 1 s). Fentanyl 40 μg/ml was introduced in a citrate buffer pH 5 (0.01 M).

The twin pulse application (i.e. the application of twin pulses composed of a first pulse of 300 V − 3 ms and followed after 1 or 0.1 s by a second pulse of 24 V − 1 sec) did not show any increase in fentanyl permeation compared to the single pulses application of five (24 V − 1.2 s) or five (300 V − 3 ms) (fig. 4, $p > 0.05$, F-test). In contrast, twin pulses increased more metoprolol permeation than single pulses (7).

Factorial Design to Study Electrical Parameters Influencing Fentanyl Permeation by Electroporation

In order to evaluate the relative influences of the voltage, duration and number of single ED pulses on fentanyl permeation, a factorial design study was performed. Factorial designs are able to handle many independent factors simultaneously. By the combination of these factors at different levels, they allow to indicate their relative significance and their various interactions in obtaining the result (15).

The factorial design performed was a fractional factorial design of three factors at three levels. The low (level −1), middle (level 0) and high (level +1) levels of the applied pulse voltage (V) were 50 V, 150 V and 250 V respectively. The corresponding transdermal voltages were only 20 V, 50 V and 60 V respectively. The low, middle and high levels of pulse duration (τ) were 133 ± 3 ms, 219 ± 6 ms, and 327 ± 6 ms respectively. Those of pulses number (N) were 5, 10 and 15 respectively. The combination of the factors levels performed are shown in table I. Table I also presents the results for each run: the cumulative quantity of fentanyl transported in the receiver compartment after 1 h and 6 h.

After logarithm transformation, the results were analysed by General Linear Model (Macintosh Systat program) (table II). The logarithm transformation was needed to obtain the homoscedasticity of the variances (Bartlett test). Table II gives a coefficient for each factor studied representing the mean

Table I. Factors Levels in Experimental Units, Number of Each Run (n) Performed, and Results for Each Run

Run	V	τ	N	n	Cumulative fentanyl transported in the receiver compartment ± sdm (ng/cm^2) after 1 h	after 6 h
1	−1	−1	−1	4	10 ± 5	106 ± 11
2	−1	+1	−1	4	25 ± 3	272 ± 30
3	+1	−1	−1	5	40 ± 7	519 ± 77
4	+1	+1	−1	5	45 ± 14	921 ± 103
5	−1	−1	+1	4	8 ± 1	226 ± 15
6	−1	+1	+1	5	25 ± 11	374 ± 39
7	+1	−1	+1	5	65 ± 29	1149 ± 121
8	+1	+1	+1	5	206 ± 90	3064 ± 436
9	0	0	0	4	49 ± 22	869 ± 59
10	−1	0	+1	4	19 ± 7	410 ± 67
11	+1	0	−1	5	50 ± 16	870 ± 156
12	0	−1	+1	4	80 ± 34	983 ± 102
13	0	+1	−1	5	33 ± 10	712 ± 115

Note: V is the applied pulse voltage, τ the pulse duration and N the pulses number. −1 is the low level, 0 the middle and +1 the high. sdm is the standard deviation of the mean.

change of the dependent variable (log of the fentanyl quantity transported) induced by an increase of the factor (V, τ, or N) from its level −1 to its level 0 or, from its level 0 to its level +1; and the significance (P value : < 0.05 is significant, < 0.01 is highly significant) of each factor, interaction term or quadratic term studied. Table II indicates that changes in all three factors have significant effects on the log of the cumulative quantities of fentanyl transported after 6 h in the receiver compartment. This table also shows the importance of some interactions forms (V * τ * N) and quadratic (V^2 and τ^2) terms on this dependent variable. The significant quadratic terms indicated the non-linear dependence of the variable with V and τ. Changes in V, τ, and N have also significant effect on the log of the cumulative fentanyl transported after 1 h in the receiver compartment, but V has the most significant effect (smallest P value) indicating that the lag time necessary to detect an increase in fentanyl permeation was principally voltage dependent (table II). Using their coefficient, the effects of the factors on increasing fentanyl transdermal permeation can be classified : the higher the coefficient, the greater the effect. The pulse voltage was the most important factor, followed by the pulses number and the pulse length.

Table II. General Linear Model Analysis

Factor	Log (Cumulative fentanyl transported in the receiver compartment after 1 h) Coefficient ±sd	P value	Log (Cumulative fentanyl transported in the receiver compartment after 6 h) Coefficient ±sd	P value
Constant	1.5 ± 0.2	0.000	2.96 ± 0.07	0.000
V	0.33 ± 0.06	0.000	0.37 ± 0.02	0.000
τ	0.13 ± 0.06	0.036	0.15 ± 0.02	0.000
N	0.13 ± 0.06	0.034	0.20 ± 0.02	0.000
V * τ	−0.07 ± 0.07	0.311	−0.03 ± 0.03	0.251
V * N	0.10 ± 0.06	0.139	0.04 ± 0.02	0.055
τ * N	0.03 ± 0.07	0.677	0.00 ± 0.02	0.870
V * τ * N	0.10 ± 0.06	0.133	0.05 ± 0.02	0.025
V^2	−0.2 ± 0.2	0.143	−0.23 ± 0.06	0.000
τ^2	0.0 ± 0.1	0.840	−12 ± 0.04	0.011
N^2	0.1 ± 0.3	0.681	0.0 ± 0.1	0.479

Note: The coefficients (± standard deviation sd) correspond to the mean change of the dependent variable (log of the fentanyl quantity transported) induced by an increase of the factor (V, τ, or N) from its level −1 to its level 0 or, from its level 0 to its level +1. P value is the significance (P value: <0.05 is significant, <0.01 is highly significant).

The response surface equation fitting the experimental points : log (cumulative fentanyl transported after 6 h in the receiver compartment) equal to the sum of the factors, interactions and quadratic terms each multiplied by its coefficient, was generated. A F-test estimating the lack of fit of the model showed that the equation was adequate. The ability of the model to predict the quantities of fentanyl transported by an electroporation application within the experimental space was tested. The experimental value of the cumulative quantities of fentanyl permeated the skin 6 h after the application of five (100 V − 135 ms) pulses was compared to the calculated value obtained from the equation. The experimental value was 258 ± 26 ng/cm^2 and the calculated value 210 ng/cm^2 indicating that the model generated a good approximation of the amount of drug transported across the skin. This model can be used to obtain response surface plots of the cumulative fentanyl transported through the skin after 6 h in term of the voltage, duration and number of pulses (Fig. 5a–b). These plots allowed visualization of the results of the analysis performed with the General Linear Model. Cumulative quantities of fentanyl transported after 6 h in the receiver compartment increased linearly with the voltage until 200 V above which it plateaued (Fig. 5a–b). They increased less strongly and not linearly with the pulse time (Fig. 5a). The effect of the number of pulses on fentanyl permeation was slight between 5 and 10 and high between 10 and 15 (Fig. 5b). The triple interaction term (V * τ * N) appeared to be important : the highest value of the cumulative quantities of fentanyl was observed when the voltage, the pulse duration and the number of the pulses were all three at their highest level (Fig. 5a–b).

This design had allowed to establish that the voltage, duration, and number of the exponentially-decaying pulses control the quantity of drug transported through the skin. As for metoprolol (7), a linear correlation appeared between the quantities of fentanyl transported through the skin and the applied electric energy of the single exponentially-decaying pulses (data not shown).

CONCLUSION

This report shows that the application of electric pulses can strongly promote transdermal delivery of fentanyl *in vitro* compared to passive diffusion through untreated skin. It also points out that the choice of the waveform ot the electric pulses is important : at the same applied energy, a few exponentially-decaying (ED) pulses increased fentanyl permeation more than a few square-wave pulses and to the same extent as the repeated application of higher voltage-shorter duration ED pulses. A factorial design showed that the voltage, duration, and number

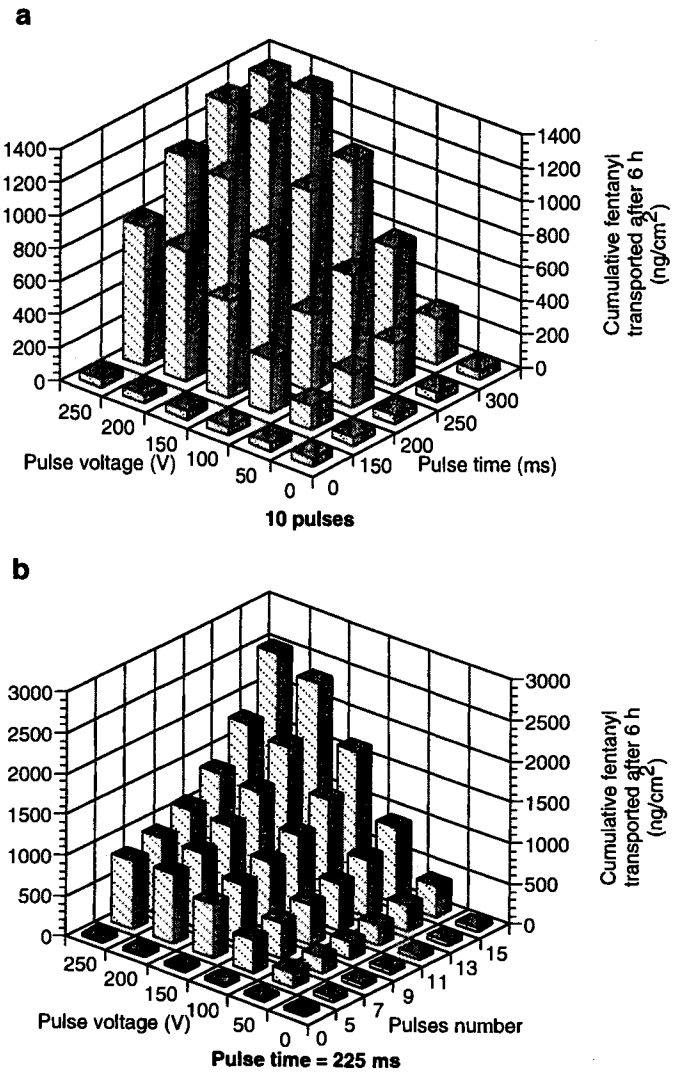

Fig. 5. Response surface plots representing calculated values obtained from the response surface equation (see text). a: Cumulative fentanyl transported after 6 h (ng/cm^2) as function of the voltage and the time of 10 exponentially-decaying pulses. b: Cumulative fentanyl transported after 6 h (ng/cm^2) in term of the voltage and number of the 225 ms exponentially-decaying pulses. Fentanyl 40 μg/ml was introduced in a citrate buffer pH 5 (0.01 M).

of ED pulses allowed control of the quantity of drug transported through the skin.

The final objective of this research was to assess the potential of electroporation for transdermal delivery of fentanyl. The effects of electric parameters on fentanyl permeation are reported in the present paper—physicochemical parameters, mechanisms and *in vivo* studies will be discussed in second paper. The results presented here indicate that skin electroporation could be a good way to improve the transdermal diffusion system of fentanyl: fentanyl permeation is enhanced by high voltage pulses, the quantity of drug delivered can be controlled by the choice of the voltage, pulse duration and number of the pulses. In addition, whereas in our *in vitro* experimental conditions skin electroporation and iontophoresis led to the same permeation profile, preliminary *in vivo* data suggested that skin electroporation could decrease dramatically the lag time necessary to observe the pharmacological effect of fentanyl in comparison to diffusion or even iontophoresis. Transdermal drug delivery by electroporation could be very promising to obtain a fast analgesic effect of the drug by a non parenteral route.

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REFERENCES

1. J. Hadgraft, and R. H. Guy (Eds), *Transdermal Drug Delivery: Developmental Issues and Research Initiatives,* Marcel Dekker Inc (1989).
2. R. Guy (Ed) Iontophoresis *Adv. Drug Delivery Rev.* **9**:119–317 (1992).
3. D. Chang, B. Chassy, J. Saunders, A. Sowers (Eds). *Guide to Electroporation and Electrofusion,* Academic Press Inc. (1992).
4. M. R. Prausnitz, V. G. Bose, R. Langer and J. C. Weaver, Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery. *Proc. Natl. Acad. Sci. USA* **90**:10504–10508 (1993).
5. R. Vanbever, N. Lecouturier and V. Préat, Transdermal delivery of metoprolol by electroporation, *Pharm. Res.,* **11**:1657–1662 (1994).
6. D. B. Bommannan, J. Tamada, L. Leung and R. O. Potts. Effect of electroporation on transdermal iontophoretic delivery of Luteinizing Hormone (LHRH) in vitro. *Pharm. Res.* **11**:1809–1814 (1994).
7. R. Vanbever and V. Preat, Factors affecting transdermal delivery of metoprolol by electroporation, *Bioelectrochem. and Bioenerget.* **38**:223–228 (1995).
8. V. Préat and R. Vanbever, Transdermal drug delivery by electroporation, in *Prediction of percutaneous penetration* vol 4 (in press).
9. R. Vanbever, D. Skrypczak, A. Jadoul and V. Préat, Comparison of skin electroperturbations induced by iontophoresis and electroporation, in *Prediction of percutaneous penetration* vol 4 (in press).
10. U. Pliquett and J. C. Weaver, Electroporation of human Skin: simultaneous measurement of changes in the transport of two fluorescent molecules and the passive electrical properties, *Bioelectrochem. and Bioenerget.* (in press).
11. J. R. Varvel, S. L. Shafer, S. S. Hwang, P. A. Coen and D. R. Stanski, Absorption characteristics of transdermally administered fentanyl, *Anesthesiology,* **70**:928–934 (1989).
12. S. Thysman, C. Tasset and V. Préat. Transdermal iontophoresis of fentanyl: delivery and mechanistic analysis. *Int J. Pharm.* **101**:105–113 (1994).
13. S. Thysman and V. Préat. In vivo iontophoresis of fentanyl and sufentanil in rats: pharmacokinetics and acute antinociceptive effects. *Anesth. Analg.,* **77**:61–66 (1993).
14. M. P. Rols and J. Teissié, Electropermeabilization of mammalian cells: quantitative analysis of the phenomenon, *Biophys. J.,* **58**:1089–1098 (1990).
15. Morgan C. *Chemometrics: Experimental design,* John Wiley & Sons (1991).