

Factors affecting transdermal delivery of metoprolol by electroporation

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Abstract

Electroporation, i.e. the creation of transient enhanced lipid membrane permeability using high intensity electric field pulses, can be used to promote transdermal drug delivery. Compared with diffusion through untreated skin, the application of electrical pulses allows us to obtain an up to 1000-fold increase in the quantities of metoprolol transported through the skin in an *in vitro* model. The present study shows that, as the voltage of five pulses of 620 ms increased from 24 to 250 V, the quantities of metoprolol permeating through the skin increased approximately in proportion to the square of the voltage. From 74 to 250 V, these quantities increased linearly with the voltage. The threshold of the voltage necessary to detect some increase in metoprolol permeation was around 24 V for these electrical conditions. As the pulse duration of five single pulses of 100 V varied from 80 to 700 ms, the quantities of metoprolol transported increased linearly. The threshold of the pulse time was approximately 80 ms for these electric conditions. The combination of these results indicated that the quantities of metoprolol that permeated through the skin was in direct proportion to the electrical energy of the pulses. The twin pulse appeared to be interesting to enhance metoprolol permeation only when the second pulse had a voltage of 24 V. The application of a low number of low voltage – long duration pulses was more efficient than the application of a high number of high voltage – short duration pulses to promote transdermal permeation of metoprolol by electroporation. These results suggest that the threshold voltage and threshold duration of the pulse are critical for transdermal permeation. As for cells, electroporabilization of the skin could be a two-steps process: one step of the induction of a transient permeated structure and another a step of the maintenance or expansion of these structures and/or electrophoretic movement.

Keywords: Transdermal delivery; Metoprolol; Membrane permeabilization

1. Introduction

Transdermal drug delivery is an interesting alternative to the conventional routes of administration, such as oral or injectable routes. However, the feasibility of passive transdermal delivery is severely restricted by the drug size, charge and required dosage, as a result of the low permeability of the skin [1]. Therefore, the number of candidates for passive transdermal drug delivery still remains small and is limited to small, lipophilic, uncharged and potent drugs.

Electroporation is a phenomenon in which the membranes of cells or lipid bilayers exposed to high intensity electric field pulses are temporarily destabilized and permeabilized. In recent years, this has been recognized as a powerful method of transporting macromolecules, such as DNA or proteins, into cells [2].

The barrier properties of the skin are attributed primarily to the multilamellar lipid domains of the stratum corneum. Electroporation of the skin has recently been reported: transient changes in tissue permeability resulting from the electroporation of the stratum corneum's intercellular lipid bilayers cause transdermal passage of molecules, which increases up to three or four orders of magnitude [3–8].

Recently [8], we have evaluated metoprolol (a β blocker with a pK_a value of 9.7, a molecular weight of 267 and $K_{oct/water} = 0.6$) permeation through full-thickness hairless rat skin *in vitro*, following electroporation with an exponentially decaying pulse. The basic electrical parameters that affect metoprolol permeation and the mechanisms implied in transdermal delivery by electroporation were elucidated. Raising the number of twin pulses from one to 20 increased the drug transport. A single pulse (100 V for 620 ms) seemed to be as effective as twin-pulse application (2200, 1100 or 300 V for 3 ms; followed after 1 s by 100 V for 620 ms). We also showed that the control of the

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pulse voltage (24–450 V) and the pulse time (80–700 ms) allowed us to control the quantity of drug delivered through the skin. The mechanisms of transdermal metoprolol delivery by electroporation involved both a transient increase in permeability and an electrostatic repulsion. A drug reservoir was also generated in the skin during pulse application, even though the pulses were very short [8].

The aim of this study was to perform a quantitative analysis of the transdermal permeation of metoprolol by electroporation. The interaction between the pulse voltage, pulse duration and pulse energy was investigated.

2. Materials and methods

The experimental methods have been described previously [8]. Briefly, the *in vitro* model was a horizontal cell made of two chambers separated by an abdominal hairless rat skin. The upper (donor) compartment contained metoprolol tartrate (10 mg ml⁻¹) in phthalate buffer of pH 3 (0.01 M), resulting in a final pH value of 4. The receptor compartment was filled with a phosphate buffer (0.024 M) of pH 7.4 isotonicized with glucose (0.151 M). Samples of solution (0.3 ml) were taken from the receptor compartment at regular intervals up to 4 h after the pulses, and were replaced with an equal volume of the drug-free buffer [9,10].

A pair of platinum electrodes of area 1 cm² separated by 1 cm were immersed in the solutions (the anode was in the donor compartment and the cathode in the receptor compartment). They were connected to the electroporation device Easyject Plus[®] (Equibio, Seraing, Belgium). The electric pulse is an exponentially decaying capacitive discharge pulse. 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. This depends on the electrical circuit resistance (composed of the shunt resistance of the Easyject Plus[®] and the cell diffusion resistance during the pulse) and the capacity of the electroporation device:

$$\tau = (\text{resistance}) \times (\text{capacity}).$$

The electroporation system allows us to modify the pulse time, by modifying the ‘‘timing resistance’’ and the capacity of the electroporation apparatus. During a pulse, the resistance of the diffusion cell was calculated by taking into account the pulse length, the capacity and the shunt resistance. The Easyject Plus can generate ‘‘high voltage’’ (HV) pulses and/or ‘‘low voltage’’ (LV) pulses. The HV pulses of 300 V were generated with a capacitance of 25 μ F and a shunt resistance of 329 or 99 Ω . The LV pulses of 250, 200, 150, 100, 74, 50 or 24 V were generated with a resistance of 2310 Ω and capacitance of 3000 μ F, to obtain a pulse time as long as possible. The Easyject Plus can be programmed to generate either single pulses (HV or LV) or twin pulses consisting of an initial HV pulse, an

interpulse delay (1 s) and a second LV pulse. Unless otherwise mentioned, the single or twin pulses were separated by 1 min.

The voltages are expressed as applied values and not transdermal values. The electrical energy of the pulses applied is given by

$$n \tau (V_i^2 - V_f^2) / 2 R_{\text{cell}}$$

where, n is the number of the electrical pulses, τ is the pulse duration, V_i is the initial voltage of the pulse, V_f is the final voltage of the pulse (8 V for the LV pulses and 34 V for the HV pulses) and R_{cell} is the resistance of the diffusion cell during the pulse. The results are expressed as the means \pm the standard error of the means ($n = 3$ –6).

3. Results

3.1. Pulse voltage

To check whether or not the voltage (V) of the pulse could affect the transdermal permeation of metoprolol, five single LV pulses (2310 Ω , 3000 μ F and 620 ms) with a voltages of 24, 50, 74, 100, 150, 200 and 250 V were applied; the corresponding transdermal voltages were only $\pm 25\%$ of these values. These electric pulses did not cause visible skin damage [8].

The pulse time was slightly longer than 620 ms for the pulses of 74, 50 and 24 V; in contrast, for the pulse of 250 V, the pulse time was slightly shortened. This showed that the pulse time and the skin resistance drop during the pulse were dependent on the pulse voltage. The resistance of the diffusion cell during the pulse dropped from 17 ± 2 k Ω before the pulse to approximately 220 Ω during the pulse for voltages of 100 V or more, while it dropped less for voltages below 100 V; to 370 ± 41 Ω for a pulse of 50 V, for example.

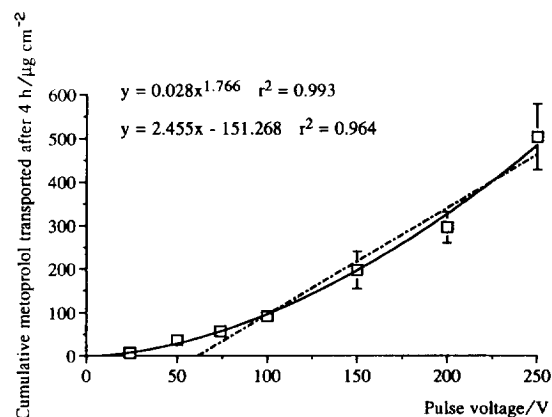


Fig. 1. Plot of cumulative quantities of metoprolol after 4 h vs. the voltage of the five LV pulses applied: $y = 0.028x^{1.766}$, $r^2 = 0.993$; $y = 2.455x - 151.268$, $r^2 = 0.964$; $C = 3000$ μ F; $R = 2310$ Ω ; 620 ms [8].

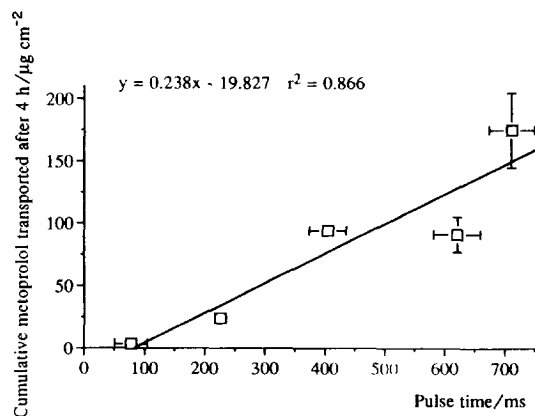


Fig. 2. Plot of cumulative quantities of metoprolol after 4 h vs. the pulse time of the five LV pulses (100 V) with different pulse times: 78 ± 27 ms, obtained with $C = 300 \mu\text{F}$ and $R = 2310 \Omega$; 226 ± 3 ms, obtained with $C = 3000 \mu\text{F}$ and $R = 99 \Omega$; 405 ± 31 ms, obtained with $C = 1950 \mu\text{F}$ and $R = 2310 \Omega$; 621 ± 39 ms, obtained with $C = 3000 \mu\text{F}$ and $R = 2310 \Omega$; 711 ± 38 ms, obtained with $C = 3000 \mu\text{F}$ and $R = \infty$ [8]. $y = 0.238x - 19.827$, $r^2 = 0.866$.

Fig. 1 shows that, above 74 V, the quantities of metoprolol that permeate through the skin after 4 h increased linearly with the voltage (correlation coefficient, 0.964), indicating that the voltage drop of the pulse could allow us to control transdermal administration by electroporation [8]. By taking into account all the voltages (24–250 V), the quantities of metoprolol transported after 4 h were approximately in direct proportion to the square of the voltage (voltage exponent, 1.766; correlation coefficient, 0.993) [11]. The threshold of the voltage necessary to detect some increase in metoprolol permeation (around 24 V) might be affected by the pulse duration and the number of the pulses [12].

3.2. Pulse time

We then studied the effect of the pulse time τ on the transdermal permeation of metoprolol. We monitored the metoprolol that permeated through the skin after the application of five single pulses of 100 V with a durations of 78, 226, 405, 621 and 711 ms. As shown in Fig. 2, the cumulative quantities of metoprolol transported after 4 h increased linearly with the pulse time (correlation coefficient, 0.866); therefore, the pulse time can also control drug permeation by electroporation, but to a lesser extent than can the voltage—at least at 100 V [8]. The resistance of the diffusion cell dropped during the pulse to around 230Ω . This value increased slightly with the inverse of the pulse time. The threshold of the pulse time necessary to detect some increase in the metoprolol that permeated the skin was approximately 80 ms for five applications of pulses of 100 V. This threshold value might be—similarly to the voltage threshold—affected by the other electrical parameters, such as the pulse voltage and the number of the pulses.

3.3. Electric energy

The present study shows that the quantities of metoprolol that permeate through the skin after 4 h increase with the square of the voltage and with the pulse duration. Combining these results, we suggest that to evaluate the metoprolol permeation, we can say that the cumulative metoprolol transported after 4 h (in micrograms per square centimeter) is given by $kV^2\tau$, where k is a constant determined by electrical conditions other than the pulse voltage V and pulse duration τ . The term $kV^2\tau$ is equivalent to the electrical energy of an electric current. Therefore, we propose that the cumulative quantity of metoprolol transported after 4 h is directly proportional to the electrical energy of the pulses.

To confirm this hypothesis, we plotted the cumulative quantities of metoprolol from the study of the effects of the pulse voltage and pulse duration, in terms of the electrical energy of the pulses (Fig. 3). It can be seen that a linear correlation exists between the quantities of metoprolol transported after 4 h and the electrical energy (correlation coefficient, 0.967).

A similar result was obtained by Okino et al. [11] in electrical impulse chemotherapy. They examined the relationship between the tumoricidal effect and the electrical variables of in vivo electrical impulse chemotherapy. Rats subcutaneously inoculated with hepatocellular carcinomas were given a single HV electrical impulse of varying voltage and duration, 30 min after an intramuscular injection of bleomycin. As the voltage (V) was increased from 0 to 5 kV, the tumoricidal effect (E) increased in proportion to the square of the voltage. As the pulse duration (D) was increased from 2.5 to 5.8 ms, the tumoricidal effect increased in direct proportion to D . Combining these results yielded the formula $E = kV^2D$, which indicates that the tumoricidal efficacy was proportional to the applied electrical energy.

A present study in our laboratory with another model compound has also shown a relationship between the electrical energy of the pulses and the drug permeation

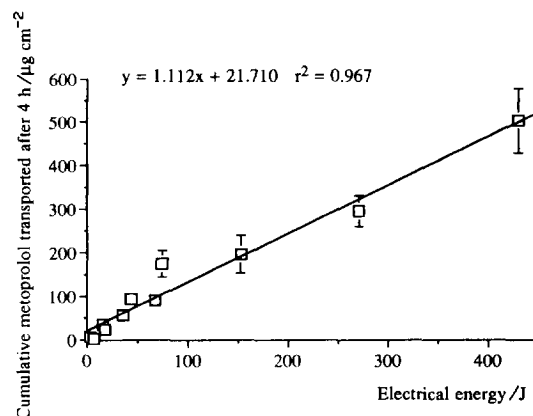


Fig. 3. Plot of cumulative quantities of metoprolol after 4 h vs. the electrical energy of the pulses applied: $y = 1.112x + 21.710$, $r^2 = 0.967$.

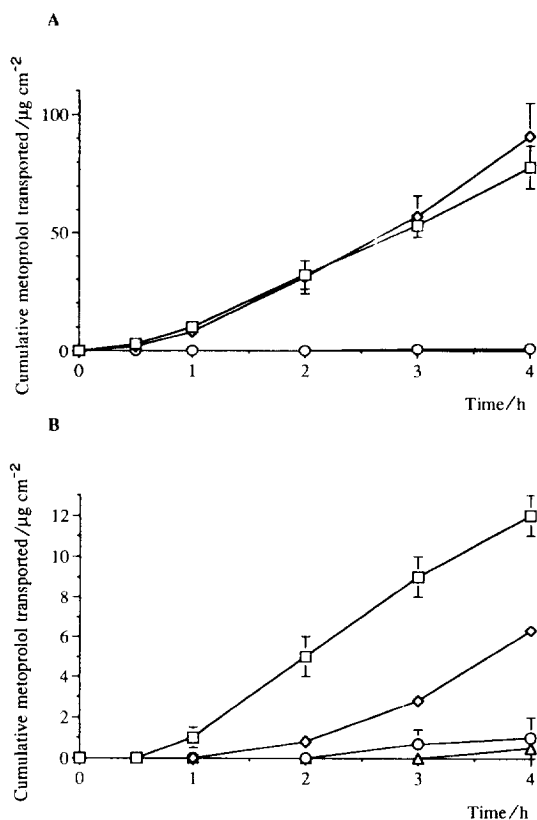


Fig. 4. (A.) Plot of the cumulative quantities of metoprolol vs. time after five sets of pulses of 300 V and 100 V (□); five pulses of 100 V (◇) and five pulses of 300 V (○) (HV pulse (300 V) $C = 25 \mu\text{F}$, $R = 329 \Omega$, $3.1 \pm 0.1 \text{ ms}$; LV pulse (100 V) $C = 3000 \mu\text{F}$, $R = 2310 \Omega$, $621 \pm 39 \text{ ms}$). (B) Plot of the cumulative quantities of metoprolol vs. time after five sets of pulses of 300 V and 24 V (□); five pulses of 24 V (◇); five pulses of 300 V (○); and after passive diffusion (Δ) (HV pulse (300 V) $C = 25 \mu\text{F}$, $R = 329 \Omega$, $3.1 \pm 0.1 \text{ ms}$; LV pulse (24 V) $C = 3000 \mu\text{F}$, $R = 2310 \Omega$, $1061 \pm 93 \text{ ms}$ for the single pulse and $899 \pm 13 \text{ ms}$ for the twin pulse).

[13]. A relationship could then exist between the electrical energy of the pulses and the transdermal permeation of a drug. Therefore, the energy of the pulses could be a means to control transdermal delivery by electroporation, at least when the pulse duration is greater than the pulse threshold.

4. Twin pulse vs. single pulse

The Easyject plus can generate either a single-pulse or a twin-pulse signal. According to Klenchin et al. [14], the initial HV pulse could create a set of electropores and start the electrophoretic drift movement; the second LV pulse could maintain this movement and lengthen the life of the electropores.

To compare the efficiency of twin pulses and single pulses, the permeation of metoprolol was evaluated after five twin pulses (300 V – for 3 ms, followed after 1 s by a LV pulse of 100 V for 620 ms) or five single LV (100 V for 620 ms) pulses were applied. As shown in Fig. 4(A), no significant differences were observed when the first pulse of the twin pulses was suppressed. The single pulse

was as efficient as the twin pulse for promoting metoprolol permeation, indicating that twin-pulse application was not necessary. We showed that a long pulse (620 ms) at a low voltage (100 V) was sufficient to “electropore” the skin. It was more efficient than an HV pulse (300 V) with a short pulse time (3 ms) for promoting metoprolol permeation, proving again that the pulse duration is a critical factor to control drug permeation [8]. The resistance of the diffusion cell dropped from $17 \pm 2 \text{ k}\Omega$ before the pulses to approximately 197 ± 9 and during the first pulse of the twin pulse and approximately $228 \pm 15 \Omega$ during the second pulse. These values are very close, indicating that a similar level of skin conductance was reached by each pulse of the twin pulse. We have obtained the same result with twin pulses composed of a first pulse of 1100 or 2200 V for 3 ms and the same second LV pulse (100 V for 620 ms) [8].

However, it could be interesting to use the twin pulse to enhance metoprolol permeation when the second long LV pulse has a voltage below 100 V, for which the skin conductance could still increase ($R_{\text{cell}} > 220 \Omega$). To check this hypothesis, we monitored the metoprolol permeation through the skin after the application of five single pulses of 24 V (1060 ms) alone, or preceded 1 s before by an HV pulse (300 V for 3 ms). These voltages were chosen, because a difference exists between their drop in the resistance of the diffusion cell during the pulse: $197 \pm 9 \Omega$ for the pulse of 300 V and $423 \pm 70 \Omega$ for the pulse of 24 V.

Fig. 4(B) shows that, under these conditions, the twin-pulse application was more efficient than the single-pulse

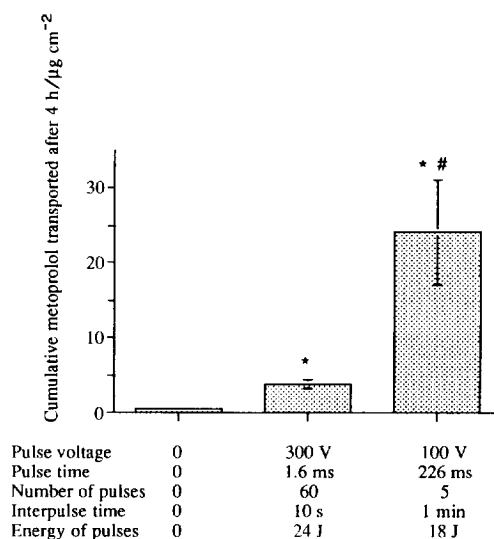


Fig. 5. Cumulative quantities of metoprolol transported in the receptor compartment 4 h after passive diffusion (first column), the application of 60 pulses of 300 V for 1.6 ms (second column; interpulse time 10s; energy of pulses, 24 J and the application five pulses of 100 V for 226 ms* (third column; interpulse time, 1 min; energy of pulses, 18 J); $p < 0.05$ vs. diffusion; #, $p < 0.05$ vs. 60 (300 V for 1.6 ms). HV pulse (300 V) $C = 25 \mu\text{F}$, $R = 99 \Omega$, $1.56 \pm 0.03 \text{ ms}$; LV pulse (100 V) $C = 3000 \mu\text{F}$, $R = 99 \Omega$, $226 \pm 3 \text{ ms}$.

application (two fold increase in cumulative quantities of metoprolol after 4 h). When the pulse of 24 V was preceeded by the pulse of 300 V, its pulse time was shortened slightly to 900 ms, which means, that the cell resistance drops during the pulse of 24 V decreased slightly more (to $344 \pm 6 \Omega$ instead of 423Ω), indicating that the effects of the first pulse (300 V) were not completely reversible and that it enhanced the effects of the second LV pulse.

5. High number of HV–short duration pulses and low number of LV–long duration pulses

We previously showed that increasing the number of the pulses (100 V for 620 ms) increased the metoprolol permeation [8]. However, to evaluate the ability of the application of a high number of HV–short duration pulses to promote the transdermal delivery of metoprolol, we have applied 60 HV (300 V for 1.6 ms) pulses with an interpulse delay of 10 s [5].

We obtained $3.8 \pm 0.6 \mu\text{g cm}^{-2}$ of metoprolol transported through the skin after 4 h. These pulses correspond to an electrical energy of $23.8 \pm 0.3 \text{ J}$. In comparison, the application of five LV (100 V for 226 ms) pulses corresponds to an energy of $17.6 \pm 0.7 \text{ J}$ and leads to a sixfold greater cumulative quantity of metoprolol transported in the receptor compartment after 4 h ($24 \pm 7 \mu\text{g cm}^{-2}$) (Fig. 5) [8].

Therefore, the application of a low number of LV–long duration pulses seems to be more efficient than the application of a high number of HV–short duration pulses for promoting transdermal permeation of metoprolol by electroporation. This could be explained by the fact that the pulse of (300 V for 1.6 ms) has sufficient voltage but a pulse duration that is shorter than the threshold (which is in the region of 80 ms). Therefore, this pulse is inadequate to enhance metoprolol permeation, except with a large increase in the number of pulses.

Furthermore, for the clinical application of drug delivery by electroporation, it would be better to decrease the number of the pulses to shorten the treatment and to decrease the number of electrically induced muscle contractions. It would also be better to decrease the voltage of the pulses to reduce the importance of this contraction (the muscle contraction increases with the voltage; data not shown).

6. Discussion and concluding remarks

Compared with diffusion through untreated skin, the application of electrical pulses allows us to obtain an up to 1000-fold increase in the quantities of metoprolol transported through the skin in an *in vitro* model. This study has shown that, as the voltage of five pulses of 620 ms

increased from 24 to 250 V, the quantities of metoprolol that permeated through the skin increased approximately in proportion to the square of the voltage. From 74 to 250 V, these quantities increased linearly with the voltage. The threshold of the voltage necessary to detect some increase in metoprolol permeation was around 24 V for this electrical application. As the pulse duration of five single pulses of 100 V varied from 80 to 700 ms, the quantities of metoprolol transported increased linearly. The threshold of the pulse time necessary to detect some increase in metoprolol permeation was approximately 80 ms for these electrical conditions. The combination of these results indicated that the quantities of metoprolol permeating through the skin was in direct proportion to the electrical energy of the pulses.

The twin pulse appeared to be interesting for enhancing the metoprolol permeation when the second pulse had a voltage of 24 V (Fig. 4(B)). The application of a low number of LV–long duration pulses was more efficient than was the application of a high number of HV–short duration pulses (pulse duration below the threshold of 80 ms) for promoting the transdermal permeation of metoprolol by electroporation.

These results suggest that the threshold voltage and threshold duration of the pulse are critical to obtain an increase in metoprolol permeation. Below the voltage and duration thresholds, there is little metoprolol transport. In parallel to what was previously reported for cell electroporation by Rols and Teissi   [12], electroporation of the skin could be a two-step phenomenon, consisting of a step of the induction of transient permeated structures for field intensities greater than the threshold and another step of the maintenance or expansion of these structures and/or electrophoretic movement [8], depending on the pulse number and duration [12]. The data reported here confirm this hypothesis.

Indeed, pulses of 300 V for 3 ms are sufficient to permeabilize the skin but are too short (less than the threshold duration) to maintain or expand the “pores” or “permeated structures”, and/or to drive the electrophoretic movement [8] (Fig. 4). However, when the number of pulses is increased, metoprolol transport is increased (Fig 5). The pulses of 100 V for 620 ms are large enough to create the permeated structures and long enough to expand these structures and/or drive the electrophoretic movement (Figs. 1,2 and 4). The pulses of 24 V are not very efficient for increasing the skin permeability, unless preceeded by a pulse of 300 V for 3 ms, which can “electropermeabilize” the skin more. Transdermal delivery of many drugs could be possible at therapeutic levels thanks to electroporation [3–8].

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