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Transdermal delivery of timolol by electroporation through human skin

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Abstract

The purpose was to achieve therapeutic fluxes of timolol by transdermal delivery using skin electroporation. The transdermal transport of timolol through human stratum corneum was studied in three compartment diffusion cells. The electrodes, buffer composition and pulse conditions were optimized. Timolol maleate concentration in the donor compartment was 40 mg/ml. Square wave pulses were applied. Electroporation enhanced the transdermal transport of timolol by 1–2 orders of magnitude as compared to passive diffusion. Even though the current application lasted for only 10 s, the transdermal transport remained high after pulsing for at least 6 h. Higher fluxes were obtained with Pt electrodes close to the skin and a phosphate buffer. 10 pulses of 400 V–10 ms were more efficient than 10 low voltage–long duration pulses. Therapeutic fluxes of timolol (>50 $\mu g/cm^2$ per h) through human stratum corneum were achieved by electroporation.

Keywords: Transdermal transport; Electroporation; Human skin; Timolol

1. Introduction

Transdermal drug delivery is a viable administration route for drugs with a low oral bioavailability. However due to the barrier function of the skin, the transdermal transport of most drugs by passive diffusion is very low and is not sufficient to achieve therapeutic levels. Hence, different approaches have been developed to overcome this skin barrier and to have a better control of drug transport across the skin. These involve chemical and physical methods based on two strategies: enhancing skin permeability and providing a driving force acting on the drug. Chemical penetration enhancers have been shown to increase transdermal transport of both small lipophilic and hydrophilic compounds mainly by affecting the stratum corneum structure. An electric field can also be used to enhance transdermal delivery. Iontophoresis employs a low density electrical current (from 0.1 to 0.5 mA/cm^2) applied for a relatively long period (from minutes to hours). Electroporation consists in the application of short (from 100 μs to less than 1 s) high voltage pulses (from about 100 to 1000 V) [1,2]. While iontophoresis acts primarily on the drug by electrorepulsion and electroosmosis, involving skin structural changes as a secondary effect, electroporation acts

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directly on the skin inducing change in tissue permeability.

Electroporation is a phenomenon in which the membranes of cells or lipid bilayers exposed to high voltage electric pulses are temporarily destabilized and permeabilized. Although the molecular mechanism by which fields interact with lipid bilayers is still incompletely understood, it is generally accepted that some of the rearrangements consist of temporary aqueous pathways, with the electric field playing the dual role of causing pore formation and providing a local driving force for ionic and molecular transport (for review, see Ref. [3]). Application of high voltage electric field pulses has been shown to increase transport across and into the skin, of hydrophilic molecules, neutral or highly charged compounds, and macromolecules with a molecular weight cut off larger than 40 kDa [4]. Topical delivery of small dyes, drugs, oligonucleotides, DNA plasmids and antigens can also be enhanced by skin electroporation (for review, see Ref. [5]). These data suggest that skin electroporation could be used to expand the range of compounds delivered transdermally or topically to hydrophilic, charged and even macromolecular compounds.

Timolol is a hydrophilic β-adrenergic blocking agent. It is non-cardioselective without intrinsic sympathomimetic and membrane stabilising activity. Timolol is effective against hypertension, arrhythmias and angina pectoris as well as for the secondary prevention of myocardial infarction. Due to its high hepatic first pass effect (50%), its short half-life (4 h) (AHFS Drug information, Blocadren manufacturer's labelling) and the need to achieve constant plasma levels, transdermal delivery could be a potential alternative to oral delivery. In humans, the oral dose of timolol maleate varies between 10 and a maximum of 60 mg/day. Taking in account an oral bioavailability of 50% and a daily oral dose of timolol of 22 mg (i.e., 30 mg of timolol maleate), transdermal flux of approximatively 500 μg/h or 50 $\mu g/cm^2$ per h for a 10-cm^2 patch should be delivered. Several reports have shown that significant transdermal transport of timolol could be achieved. Transdermal application of timolol maleate has been reported to produce an appropriate β-blocking effect in rodents [6]. Its transport by passive diffusion from aqueous solution is in the range of 5 $\mu g/cm^2$ per h. Using water-activated, pH-controlled silicone reservoir devices for transdermal delivery of timolol in volunteers, steady-state plasma concentrations could be reached with a considerable inter-individual variability due to the high fractional skin control in timolol delivery [7]. The timolol patches applied for 81 h were well tolerated: skin irritation induced by the combination of timolol with occlusion was mild [8]. Recently, transdermal iontophoresis of timolol maleate has been studied: iontophoresis increased the transport, reduced the lag-time and allowed control of the delivery of the drug [6,9,10]. The steady state flux of timolol found by Kanikkannan et al. [6] through human epidermis after 2 h of iontophoresis with a current density of 0.375 mA/cm^2 was 10 μg/cm per h. The initial concentration of timolol maleate in the donor solution was 1 mg/ml. As an alternative to iontophoresis applied for hours or passive diffusion with very large patches, the potential of electroporation for transdermal delivery of timolol was investigated. Assuming that application of short pulses induces an increase in drug transport and controls drug transport [1–5], the aim of this report was to evaluate if therapeutic fluxes of timolol could be achieved using electroporation. An in vitro three-compartment diffusion cell with human stratum corneum was used in order to mimic the in vivo situation, i.e., both electrodes were placed on the outer side of the stratum corneum. Since the barrier to drug transport is located mainly in the stratum corneum [11], human stratum corneum was used as a model of skin barrier. Square wave pulses were selected to achieve reproducible constant pulse voltage and duration. To optimize timolol transport, the influence of electrical factors, buffer composition and electrodes were studied.

2. Materials and methods

2.1. Materials

Timolol maleate was purchased from Sigma (St. Louis, MO, USA). The salts used to prepare the buffers and acetic acid (99–100%) were supplied by VWR (Leuven, Belgium). Platinum was obtained from Aldrich (Bornem, Belgium) and was >99.99% pure. HPLC-grade acetonitrile (J.T. Bakker, Deven-

ter, The Netherlands) was used as a solvent in the HPLC analysis. All solutions were prepared with ultrapure water (Sation 9000, conductivity 0.1 μS/cm, VWR, Leuven, Belgium). Dialysis membranes with a molecular weight cut-off of 5 kDa (Diachema, Munchen, Germany) were used as a support [12].

2.2. Skin preparation

Human abdominal skin was obtained from plastic surgery and was prepared within 24 h. After removal of fatty tissue, the skin was dermatomed at ±300 μm (model 1993 dermatome, Robbins Instruments, Chatham, USA). To obtain isolated stratum corneum, dermatomed skin was incubated with its dermal side on paper soaked in a solution of 0.1% (w/v) trypsin (from bovine pancreas, T4665, Sigma) in 0.15 M phosphate-buffered saline (PBS) overnight at 4 °C and 1 h at 37 °C. The stratum corneum was then carefully peeled off. The stratum corneum was treated with 0.1% (w/v) trypsin inhibitor solution (type II-S from soybean, T9128, Sigma) in PBS. Then, it was washed twice with distilled water, blotted dry and stored at room temperature in a silica gel containing desiccator in N_2 in order to avoid oxidation of lipids [13].

2.3. In vitro model

Transport experiments were performed in a three-chamber continuous flow through diffusion cell. One piece of stratum corneum with a dialysis membrane used as a supporting membrane was placed between the outer chamber (anodal compartment) and the central acceptor chamber. Another piece of stratum corneum was placed between the central chamber and the other outer chamber (cathodal compartment). The central chamber was thermostated at 37 °C. The exposed area was 0.64 cm^2, the acceptor volume and the donor volume were 0.5 and 2 ml, respectively, [12,14]. The donor solutions contained 40 mg of timolol maleate per ml of buffer (anodal compartment). The acceptor chamber was filled with PBS, pH 7.4, and the cathodal compartment with 2 ml of maleate or phosphate buffer. During the experiments, both anodal and cathodal chambers were magnetically stirred at 350 rpm (Variomag, Euro-Scientific, Lint, Belgium). A pair of inert electrodes made of platinum were connected to the electroporation device. The acceptor flow rate was 6.5 ml/h and the acceptor compartment was sampled every hour. Due to the difference in skin permeability of different donors, human skin specimens from three different donors were used for each condition tested. Timolol fluxes through dialysis membrane alone were very high: 2433 and 3077 $\mu g/cm^2$ per h after passive diffusion and electroporation, respectively. Timolol concentration in the counter electrode compartment was below the limit of detection (<25 ng/ml).

2.4. Electroporation protocol

The pulse generator was a square wave pulser controlled by a PC computer (PA-4000, Pulse Agile Electroporation System, Cyto Pulse Sciences, Columbia, USA). An oscilloscope (Hewlett-Packard 54602B, Colorado Springs, USA) was placed in parallel in the electrical circuit to check the pulse waveform, the pulse voltage and the pulse duration. The square wave pulses were chosen to ensure a constant pulse duration and voltage whatever the skin resistance and donor composition. Ten pulses were selected as a reference value since one or two pulses might be ineffective, whereas hundreds of pulses might be painful for the patient. A voltage of 400 V was selected as the maximum voltage. Different pulse voltages and pulse durations were tested: 250 and 400 V; 1, 10, 20, and 200 ms. The pulse cycle was 1 s, i.e., the pulse duration plus the pulse interval was 1 s. The voltage is expressed as applied voltage and not as transdermal voltage. Platinum electrodes were used in the anodal and cathodal compartments, placed at either 20 or 4 mm from the membrane. Passive diffusion of timolol was followed for 6 h, then 10 pulses were performed and the diffusion after electroporation was evaluated for 6 h. The current through the cell system (I) was measured with the oscilloscope connected to a special output of the pulser. The resistance of the diffusion cell (R) during electroporation was determined by Ohm's law: $R = V/I$ where V is the applied pulse voltage. Transdermal voltages were estimated by calculating the ratio of the skin resistance to the total cell resistance. This ratio is equal to the ratio of the transdermal voltage to the voltage across the whole diffusion cell (applied voltage) [1]. The energy of the

electrical square wave pulses (W) applied to the solutions and skin was calculated from the equation: $W = N \cdot D \cdot V^2/R$ where N is the number of pulses, D is the pulse duration, V is the applied voltage and R is the resistance of the diffusion cell during the pulse [15,16]. The amount of charges transported was calculated as $N \cdot D \cdot V/R$ [16].

2.5. Effect of buffer composition and pH

The pH influences the charge of the drug and ions of the buffer compete with timolol for transport. Hence the effects of pH and buffer composition on timolol transport were evaluated. Three different buffer compositions were used in the outer compartments for the experiments: the phosphate buffers, pH 7.4–50 and 150 mM, were chosen to mimic physiological conditions and the maleate buffer, pH 5.0–100 mM, was chosen in order to use the same salt as timolol and a similar pH than that of the skin. In all cases the acceptor phase was maintained at pH 7.4 in PBS. The conductivity of the buffers was measured by a conductimeter (WTW, Weilheim, Germany).

2.6. HPLC analysis

For the analysis of the samples containing timolol, an HPLC method was used. Briefly the HPLC system consisted of an automatic injector, a gradient pump and a UV detector (Bio-Tek Instruments, Milano, Italy). The wavelength was 294 nm. An X-Terra RP C18 column was used (15 cm×3.0 mm, 3.5 μm, Waters, Milford, USA). The mobile phase consisted of acetonitrile–phosphate buffer (25:75, v/v). The 50 mM phosphate buffer was adjusted to pH 3.0 with pure acetic acid. The flow rate was 0.5 ml/min and the injection volume was 50 μl. Chromatography was performed at room temperature and the retention time of timolol maleate was 2.5 min. The calibration curves were linear ($r^2 > 0.999$) over the concentration range studied (5–100 μg/ml). The intra-day coefficients of variation were less than 3% and the day-to-day coefficients of variation were less than 5%.

2.7. Data analysis

Timolol fluxes were calculated from the concentrations in the collecting tubes by using the equation: $J_F = F \cdot C_a/A$ where J_F is the flux through the membrane, C_a is the concentration of timolol in the acceptor phase determined by HPLC, A is the skin area and F is the flow rate of PBS. When the flux reached steady state, this steady state flux (J_{ss}) was obtained from the linear part of the plot of fluxes versus time. From J_{ss} the permeability coefficient K_p (expressed in cm s^{-1}) can be calculated according to $J_{ss} = K_p \cdot C_{don}$, where C_{don} is the concentration of the donor solution. The enhancement factor $EF = J_{ss}(\text{electroporation})/J_{ss}(\text{passive diffusion})$ was calculated. The results were expressed as the mean±standard error of the mean. Statistical comparisons were made using the t-test. The probability value of less than 0.05 was considered to be significant.

3. Results

3.1. Effect of electrode position

In order to check if the distance between the electrodes influences drug transport, the platinum electrodes were placed at 20 and 4 mm from the membrane. The steady state fluxes of timolol through human stratum corneum were determined for passive diffusion and electroporation (Fig. 1). When the electrodes were placed at 20 mm, there was no difference between passive diffusion and electroporation fluxes (t-test, $P > 0.05$). When the electrodes were placed at 4 mm from the stratum corneum, the flux of timolol obtained with an electroporation of 10× (400 V–1 ms) (24 μg/cm^2 per h) was about five times higher than the flux obtained with passive diffusion (5 μg/cm^2 per h). There was also a significant difference between the resistances of the cell during electroporation when the distance was 20 and 4 mm: 229 and 505 Ω for 4 and 20 mm, respectively (t-test, $P < 0.01$). When the electrodes were closer, the drop in the cell resistance and the timolol flux were higher. Hence the following experiments were performed with an electrode distance of 4 mm from the stratum corneum, i.e., 16 mm for the total distance between the two electrodes (2× 4 mm+8 mm (receptor compartment)), corresponding

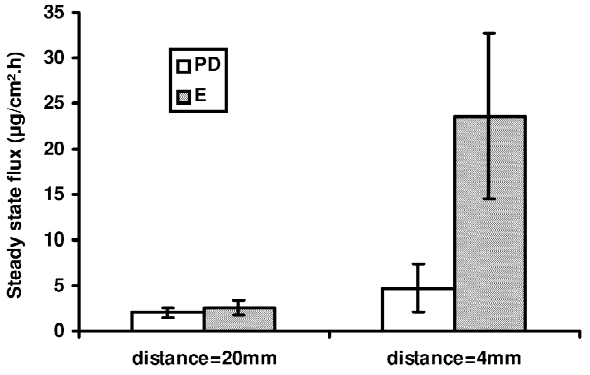

Fig. 1. Steady state fluxes of timolol by passive diffusion (PD) and electroporation (E) 10× (400 V–1 ms) for two positions of the electrodes (mean±S.E.M., $n=4$ and $n=3$ for 20 and 4 mm, respectively). Timolol maleate 40 mg/ml was introduced in a phosphate buffer, pH 7.4–50 mM.

to an electric field of 156 and 250 V/cm for a pulse voltage of 250 and 400 V, respectively.

3.2. Effect of pH and buffer composition

To control and to fix the pH of the drug solution, the use of a buffer is required, ensuring drug ionisation and avoiding the pH shift due to water electrolysis induced by electroporation with platinum electrodes. Two different buffers were tested: a maleate buffer and a phosphate buffer. The influence of the ionic strength of the phosphate buffer was also studied. The pH, the conductivity, the ionic strength of three buffers and the resistance of the cell during electroporation experiments are presented in Table 1. The phosphate 50 mM buffer was the lowest concentration required to avoid the pH shift due to water electrolysis by electroporation (data not shown). The conductivity of the maleate 100 mM buffer was similar to that of the phosphate 50 mM buffer.

The comparison of the steady state fluxes of timolol transported through human stratum corneum with the three different buffers indicates that the flux after electroporation (Fig. 2) and the enhancement factor (Table 1) were the highest for the phosphate 50 mM buffer, however there was no significant difference with the maleate 100 mM and the phosphate 150 mM buffers (*t*-test, $P>0.05$). There was no significant difference either in the drop of the cell resistance during electroporation between the phosphate 50 mM buffer and the phosphate 150 mM buffer (*t*-test, $P>0.05$) (Table 1). The transport of timolol was neither increased by the higher conductivity of the phosphate 150 mM buffer, as compared to the phosphate 50 mM buffer, nor by a lower pH as the maleate 100 mM buffer. Hence, the phosphate 50 mM buffer was chosen in the following experiments.

3.3. Effect of the pulse voltage and duration

The electrical factors affecting drug delivery by electroporation are the pulse wave form, the voltage, the pulse duration and the number of pulses [16,17]. Hence different voltages and pulse durations were tested. Medium voltage–medium duration pulses (400 V–1, 10 and 20 ms) and low voltage–long duration pulses (250 V–100 and 200 ms) were compared [18]. Transdermal voltages were found to be approximately 22% of applied voltages, corresponding to 86 and 54 V for an applied voltage of 400 and 250 V, respectively. Fluxes of timolol through human stratum corneum were determined for passive diffusion and electroporation: the comparison of fluxes and steady state fluxes obtained with different voltages and pulse durations are presented in Figs. 3 and 4. The higher fluxes (109 and 78 $\mu g/cm^2$ per h) were obtained when the electropora-

Table 1
Properties of the different buffers

Buffer	Conc. (mM)	pH	Ionic strength (mM)	Conductivity (mS/cm)	Resistance of the cell (Ω)	Enhancement factor
Maleate	100	5.0	350	6.23	222±15	18.0±6.3
Phosphate	50	7.4	125	6.71	169±16	21.8±8.3
Phosphate	150	7.4	525	17.09	151±7	15.3±4.5

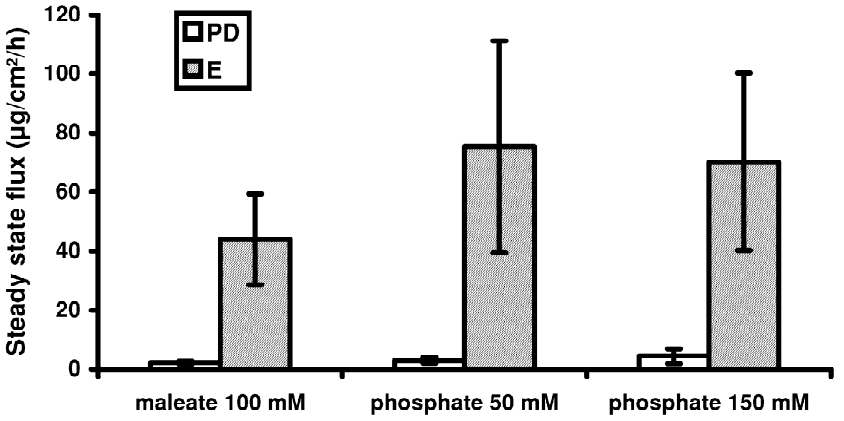

Fig. 2. Steady state fluxes of timolol by passive diffusion (PD) and electroporation (E) 10× (400 V–10 ms) through human stratum corneum. Timolol maleate 40 mg/ml was introduced in a maleate buffer, pH 5.0–100 mM, a phosphate buffer, pH 7.4–50 or 150 mM (mean±S.E.M., $n=5$ for maleate 100 mM and phosphate 50 mM, $n=4$ for phosphate 150 mM).

tion conditions were 400 V–20 ms and 400 V–10 ms, respectively. The lag-time was short: the maximum steady-state flux (109 $\mu g/cm^2$ per h) was reached within 1 h and remained constant for at least 6 h after high voltage pulses application.

The electrical energy applied to the diffusion cell and the quantity of charges transferred across the skin during the pulses were lower for medium voltage–medium duration pulses while the fluxes obtained were higher, indicating that the application of medium voltage–medium duration pulses was more efficient than the application of low voltage–long duration pulses for promoting transport of timolol by electroporation with square wave pulses.

The permeability values were determined for passive diffusion and for the different electroporation conditions tested. They are in the range of 3×10^{-8} cm s^{-1} for passive diffusion, in accordance with literature values (2×10^{-8} cm s^{-1} [7]), and $1–7\times10^{-7}$ cm s^{-1} after electroporation (Table 2).

4. Discussion

4.1. Timolol transport using skin electroporation

Skin electroporation can increase transdermal transport up to 3 orders of magnitude with lag-times of only seconds to minutes in vitro showing that molecules rapidly respond to electric pulses. The enhancement magnitude and the onset times for transport have been shown to depend on the pulsing protocols and the physicochemical properties of the molecule being transported as well as the skin model [1,16,18]. The application of electrical pulses promoted transdermal transport of timolol through human stratum corneum in an in vitro model. Even though the application of the current lasted for less than 2 s (corresponding to 10 s of treatment in total), the maximum flux was reached within 1 h and remained high for at least 6 h, indicating that this very short current application can induce the transport of large quantities of timolol. Timolol transport could be controlled by the electrical parameters and the donor formulation: the electrode position, the buffer composition, as well as the voltage and pulse duration influenced the quantity of drug delivered through human stratum corneum. The distance between electrodes influenced drug transport. The application of medium voltage–medium duration pulses (400 V) was more efficient than the application of low voltage–long duration pulses (250 V) for promoting the transdermal permeation of timolol by electroporation using square wave pulses. When comparing short, high-voltage and long, medium-voltage pulses for the transport of sulforhodamine across human skin [18], long pulses of medium voltage were found to be more efficient in transport-

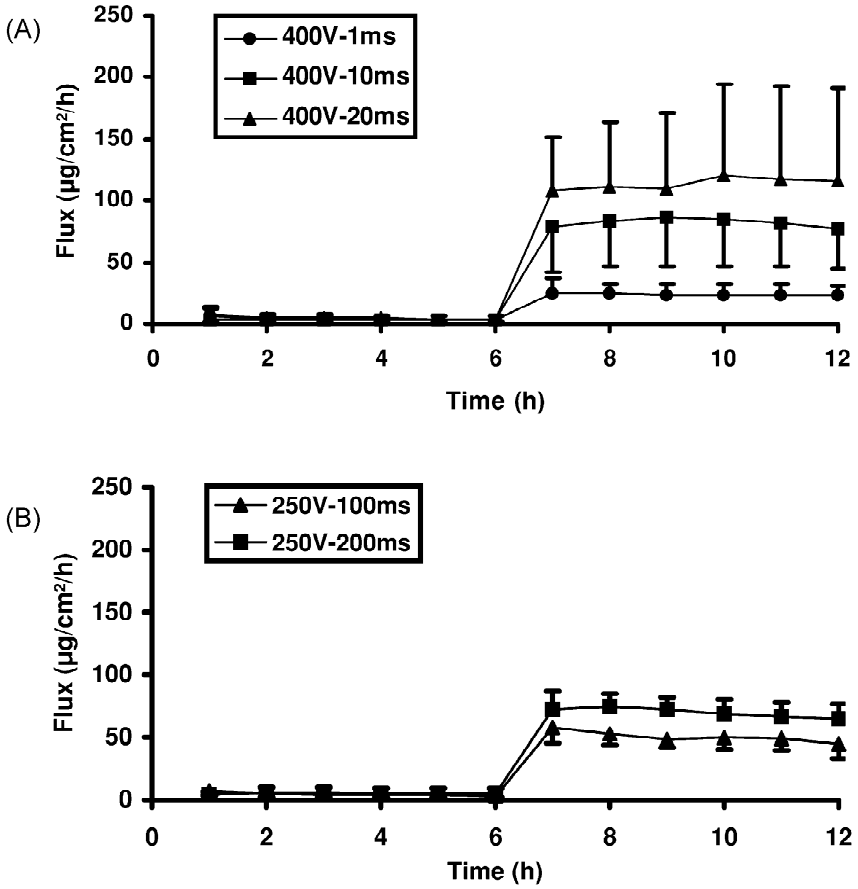

Fig. 3. Instantaneous fluxes of timolol through human stratum corneum after 6 h of passive diffusion followed by electroporation with (A) medium voltage–medium duration pulses (10× 400 V–1, 10 and 20 ms; mean±S.E.M., $n=3$, 5, 7, respectively) and (B) low voltage–long duration pulses (10× 250 V–100 and 200 ms; mean±S.E.M., $n=4$, 6, respectively). Timolol maleate 40 mg/ml was introduced in a phosphate buffer, pH 7.4–50 mM.

ing sulforhodamine by electroporation, probably due to higher electrophoresis induced by exponential-decay pulses compared to square wave pulses.

4.2. Electroporation versus passive diffusion and iontophoresis

As compared to passive diffusion, electroporation significantly enhanced the transdermal transport of timolol. Depending on the electroporation conditions and reservoir formulation, the enhancement factors varied between 5 and 25. Compared to the literature data, the enhancing effect of electroporation in the experimental conditions used is rather low [17]. For most drugs, including another β-blocker metoprolol [2,15], electroporation increases drug transport by 2–3 orders of magnitude. The reasons for this low enhancement factor of transport by electroporation could be: (i) square wave pulses, which are required to get constant parameters in patients but could decrease the contribution of electrophoresis as compared to exponentially decaying pulses [16]; (ii) the higher log *P* than metoprolol, which could lead to a decrease in drug transport as observed for the iontophoretic transport of propranolol [2], and for transport by electroporation in the presence of propranolol (Préat et al., unpublished data); (iii) the higher passive diffusion flux due to the higher log *P*

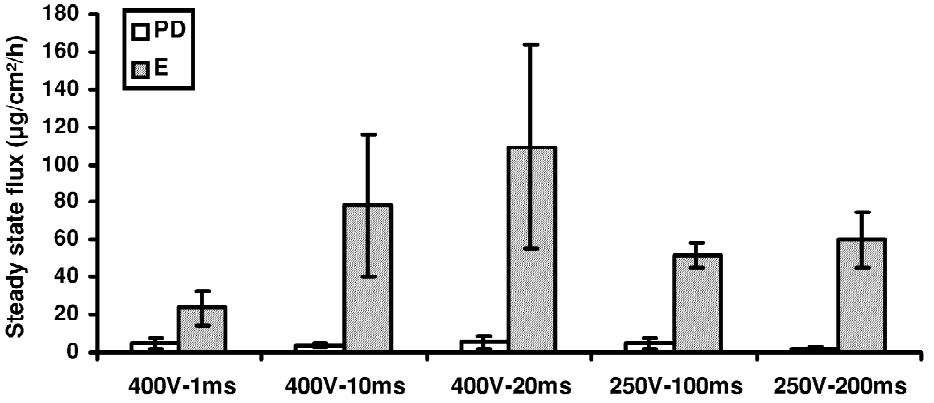

Fig. 4. Steady state fluxes of timolol through human stratum corneum obtained with different voltages and pulse durations (mean±S.E.M.; 400 V–1 ms, $n=3$; 400 V–10 ms, $n=5$; 400 V–20 ms, $n=7$; 250 V–100 ms, $n=4$; 250 V–200 ms, $n=6$). Timolol maleate 40 mg/ml was introduced in a phosphate buffer, pH 7.4–50 mM.

than metoprolol, resulting in a decrease of the enhancement ratio. Indeed, the absolute fluxes of timolol and metoprolol [2] after electroporation were in the same range of magnitude (permeability $=7\times 10^{-7}$ cm s^{-1}).

Elevated fluxes of timolol can be obtained by both iontophoresis and electroporation. However, a major difference is the application of the treatment: in iontophoresis, several minutes to hours of current application are needed, compared to seconds in electroporation.

4.3. Electroporation for transdermal delivery of timolol in humans

The main potential advantage of electroporation for a clinical use in transdermal drug delivery is the short electrical pulse treatment required. Pulse application during less than 10 s induced increased stratum corneum permeability, which lasted for hours. As mentioned previously, to deliver transdermally 11 mg of timolol per day (equivalent to a daily oral dose of 30 mg of timolol maleate), a transdermal flux of timolol 500 μg/h or 50 μg/cm^2 per h for a 10-cm^2 patch should be achieved. The in vitro data clearly demonstrate that these fluxes can be achieved using electroporation. After 10 pulses of 400 V–10 ms, timolol fluxes of 78 μg/cm^2 per h were reached with a concentration of 40 mg/ml timolol maleate. To achieve sustained release of the drug, the patch containing drug reservoir needs to be left on the skin after pulse application. The control of the dose of timolol delivered can be achieved by changing the pulse conditions. Decreasing the voltage and electric field applied, the number or the duration of the pulses will decrease timolol transport. Alternatively, the size of the patch or the drug concentration could be decreased (data not shown). Therapeutic fluxes of

Table 2
Electrical parameters, fluxes and permeability values for transport of timolol through dialysis membrane and the supported human stratum corneum at different electroporation conditions tested

Pulse voltage (V)	Pulse duration (ms)	Resistance of cell (Ω)	Energy (J)	Quantity of charges (C)	Flux (μg/cm^2 per h)	Permeability (cm s^{-1})
400	1	229	7	0.02	24±9	$(1.62\pm0.61)\times10^{-7}$
400	10	169	95	0.24	78±38	$(5.23\pm2.59)\times10^{-7}$
400	20	119	267	0.68	109±54	$(7.54\pm3.73)\times10^{-7}$
250	100	171	365	1.50	52±7	$(3.59\pm0.46)\times10^{-7}$
250	200	181	691	2.60	60±15	$(4.16\pm1.02)\times10^{-7}$

timolol were also reached with the 250 V protocols, but with a higher level of energy applied. Optimisation of high voltage protocols both in terms of transdermal molecular transport and skin tolerance should be considered. Indeed, the major concern associated with the use of electroporation is the safety issue, even though several reports indicate that the damage to the skin is very mild and reversible [5,19,20]. The prolonged enhanced permeability of stratum corneum to timolol transport after electroporation, associated with a dramatic decrease of impedance, could be explained: (i) by the changes in the stratum corneum structure; and/or (ii) as a result of skin damage and the absence of repair mechanisms in vitro. Recently, square wave pulses were shown to induce in rats a very mild and transient impairment of the transepidermal water loss. However, as expected, the pulses induced a very dramatic decrease in the impedance of the skin which lasted for hours and recovered progressively within 24–48 h [21]. The pulses applied with the current electrode design induce muscle contraction and could reach the pain threshold. Patients submitted to electrochemotherapy seem to tolerate well the application of 10× (1000 V/cm–100 μs) square wave pulses [22]. Similarly, even in sensitive areas like the penis, application of single exponentially decaying pulses of 50–80 V–3 ms was found to be acceptable by most patients [23]. To avoid this pain sensation, milder electroporation conditions (lower voltage and/or shorter pulses) could be used but drug transport might be decreased to subtherapeutic values. A more promising solution might be to improve electrode design to restrict the electrical field to the superficial layers of the skin [23,24]. Alternatively, a low number of pulses to permeabilize the skin could be combined with iontophoresis to drive the drug into the permeabilized skin.

5. Conclusion

Skin electroporation can strongly promote transdermal delivery of timolol in vitro compared to passive diffusion and therapeutic fluxes of timolol (>50 $\mu g/cm^2$ per h) through human stratum corneum were achieved with the electrical protocols used. The choice of the electrical parameters (voltage, duration and number of pulses) allows a control of the quantity of drug transported through the skin. The application of medium voltage–medium duration pulses (400 V) were found to be more efficient in terms of transport and energy required to obtain these therapeutic fluxes of timolol. Although the major concern with electroporation could be the safety issue, the advantages of electroporation, i.e., rapid and enhanced delivery and control of the dose delivered, confirm the interest of electroporation to enhance drug delivery.

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