Transdermal Delivery of Metoprolol by Electroporation

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Electroporation, i.e., the creation of transient "pores" in lipid membranes leading to increased permeability, could be used to promote transdermal drug delivery. We have evaluated metoprolol permeation through full thickness hairless rat skin in vitro following electroporation with an exponentially decaying pulse. Application of electric pulses increased metoprolol permeation as compared to diffusion through untreated skin. Raising the number of twin pulses (300 V, 3 ms; followed after 1 s by 100 V, 620 ms) from 1 to 20 increased drug transport. Single pulse (100 V, 620 ms) was as effective as twin pulse application (2200 V, 1100 V or 300 V, 3 ms; followed after 1 s by 100 V, 620 ms). In order to investigate the effect of pulse voltage on metoprolol permeation, 5 single pulses (each separated by 1 min) were applied at varying voltages from 24 to 450 V (pulse time 620 ms). A linear correlation between pulse voltage and cumulative metoprolol transported after 4 h suggested that voltage controls the quantity of drug delivered. Then, the effect of pulse time on metoprolol permeation was studied by varying pulse duration of 5 single 100 V pulses from 80 to 710 ms (each pulse also separated by 1 min). Cumulative metoprolol transported after 4 h increased linearly with the pulse time. Therefore, pulse time was also a control factor of the quantity of drug delivered but to a lesser extent than the voltage at least at 100 V. The mechanisms behind improved transdermal drug delivery by electroporation involved reversible increased skin permeability, electrophoretic movement of drug into the skin during pulse application, and drug release from the skin reservoir formed by electroporation. Thus, electroporation did occur as shown by the increased transdermal permeation, on indicator of structural skin changes and their reversibility. Electroporation has potential for enhancing transdermal drug delivery.

KEY WORDS: transdermal drug delivery; electroporation; metoprolol; skin permeation.

INTRODUCTION

Transdermal drug delivery is a useful alternative to the conventional routes of administration such as oral or injectable routes. It avoids degradation in the gastrointestinal tract and first-pass hepatic metabolism. Transdermal administration allows steady or time varying controlled delivery and improves patient compliance. However, very few drugs can be administered transdermally due to the low permeability of the skin. It is presently applicable for only a few drugs. Such molecules are typically small and relatively lipophilic. For charged, polar molecules, administration by the cutaneous route is difficult due to the intrinsic lipophilicity of the stratum corneum. Several methods have been employed to enhance transdermal delivery of such drugs (1). Chemical penetration enhancers have been extensively studied. Iontophoresis is another technique used to enhance delivery of charged and neutral polar molecules. It uses an electrical potential gradient as a driving force. However, if iontophoresis offers the possibility of systemic delivery in a controlled and effective fashion without extensive skin damage (1,2), it needs low intensity electric current application for several minutes if not hours and is limited to relatively low molecular weight drugs.

Electroporation is a phenomenon in which the membranes of cells or lipid bilayers exposed to high intensity electric field pulses are temporarily destabilised and permeabilized. In recent years, it has been recognized as a powerful method of transporting macromolecules, such as DNA or proteins, into cells. In spite of this success, the basic mechanisms of electroporation remain largely unknown. It is generally accepted, however, that the application of an electric pulse may create transient aqueous pores in the cell membrane (3).

The barrier properties of the skin are attributed primarily to the stratum corneum, the skin's outermost layer. The stratum corneum is composed of flattened dead cells filled with keratin and intercellular matrix with multilamellar lipid bilayers. It has been suggested that electroporation of the skin could promote transdermal drug delivery by creating transient changes in tissue permeability consequent to the "electroporation" (creation of transient aqueous pores) of the stratum corneum's intercellular lipid bilayers (4–10). While iontophoresis acts primarily on the drug, involving skin structural changes as a secondary effect, electroporation is expected to act directly on the skin making change in tissue permeability. Thus, occurrence of skin electroporation should be associated with increased transport, reversibility and evidence for skin structural changes (9).

The aim of this study was to investigate the influence of electroporation on the in vitro transdermal permeation of metoprolol, a β blocker with a high first pass hepatic metabolism (pKa = 9.7, MW = 267, $K_{oct/water}$ = 0.6) (11). The influence of the pulses' number, pulse type, pulse voltage, pulse time were studied in vitro using hairless rat skin. The mechanisms of transport by electroporation were also examined.

MATERIALS AND METHODS

Chemicals

(±)-Metoprolol (+)-tartrate and *dl*-(−)-propranolol hydrochloride were purchased from Sigma Chemical Company (St. Louis, MO). The salts used to prepare the buffers (for analysis), methanol (HPLC grade) and acetonitrile (HPLC grade) were obtained from UCB (RPL, Leuven, Belgium). All solutions were prepared in ultrapure water (Sation 900, Vel, Leuven, Belgium).

In Vitro Model

The in vitro model was a polycarbonate (Makrolon,

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Obra, Liège, Belgium) horizontal cell made of two chambers separated by a membrane. The surface area of the membrane exposed to the two solutions was 3 cm^2. The upper (donor) compartment contained 1.6 ml of drug solution. The receptor compartment with a capacity of 7.5 ml was continuously stirred magnetically and maintained at 37°C. A pair of platinum electrodes (platinum pure, Johnson Matthey, Brussels, Belgium) of 1 cm^2 were immersed in the solutions (if not mentioned, the anode was in the donor compartment and the cathode in the receptor compartment). The distance between each electrode was approximately 1 cm.

The experiments were carried out with male hairless rat skin (mutant Iops rat hairless; Iffa Credo, St Germain les Arbreles, France). The 2-3-month-old rats were sacrificed by ether breathing, and full-thickness abdominal skin was excised. Subcutaneous fat was removed carefully with scissors. The freshly excised skin specimens were mounted between the two half-cells of the permeation system, with the stratum corneum facing the donor compartment.

The receptor compartment was filled with a phosphate buffer (0.024 M) at pH 7.4 isotonized with glucose (0.151 M). Metoprolol tartrate (10 mg/ml) was introduced in phtalate buffer (0.01 M) at pH 3, resulting in a final pH of 4. No shift in pH due to pulsing was observed. Samples of solution (0.3 ml) were taken from the receptor compartment at regular intervals (0.5 or 1 h) up to 4 h after the pulses, and were replaced with an equal volume of the drug free buffer. When kinetics of permeation was followed, dilution of receptor compartment was taken into account. All samples were frozen until analyzed (11). The ratio of the cumulative quantities detected in the receptor compartment to the membrane area was plotted in term of time. The lag times were deduced from the linear part of the plot when it was possible. The results were expressed as means ± the standard error of the means (n = 3 to 6).

Electroporation

The electrodes are connected to Easyject Plus® (Equibio, Seraing, Belgium), an equipment used for electroporating bacteria and other cell membranes. Easyject Plus® is an electroporation system based on capacitor discharge. The voltage can vary from 3500 V to 20 V and is an exponentially decaying capacitive discharge pulse. Pulse time is defined as the time constant i.e. 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. This pulse length is measured by Easyject Plus®. It depends on the electrical circuit resistance (composed of the shunt resistance of Easyject Plus® and of the apparent cell diffusion resistance) and the capacity of the electroporation apparatus: pulse time = resistance × capacity. The electroporation system Easyject Plus® allows to modify the pulse time by modifying the "timing resistance" and the capacity of the electroporation device.

Easyject Plus® can generate "high voltage" (HV) pulse from 3500 V to 100 V with a maximum capacity of 25 μF and/or a "low voltage" (LV) pulse from 450 V to 20 V and a capacity varying from 150 to 3000 μF, the resistance varying from 99 Ω to infinity in both modes. High voltage (HV) pulses of 2200, 1100 or 300 V were generated with a 25 μF capacity and 329 Ω resistance. Low voltage (LV) pulses of 450, 400, 350, 300, 250, 200, 150, 100, 74, 50 or 24 V were generated with a 2310 Ω resistance and a 3000 μF capacity to get a pulse time as long as possible. Easyject Plus® can be programmed to generate either single pulse (HV or LV) or twin pulse consisting in a first HV pulse, and interpulse delay (1 s) and a second LV pulse. If more than one pulse was applied, they were separated by 1 min. Voltages are expressed as applied values and not transdermal values.

During a pulse, the apparent resistances of the diffusion cell, of the solutions (resistance of the cell without skin but filled with donor or receptor solutions) and then of the skin were evaluated. They were calculated by taking into account the pulse length, the capacity and the shunt resistance of Easyject Plus® and, the distance between each electrode and the skin. Transdermal voltages were evaluated by calculating the ratio of the apparent skin resistance to the apparent total cell resistance. This ratio is equal to the ratio of the transdermal voltage to the voltage across the whole diffusion cell (applied voltage) (9).

Analysis of Metoprolol

The drug concentration was determined by a reversed-phase high-performance liquid chromatographic method. A column of octadecylsilane (μBondapak C_{18}; 30 cm × 3.9 mm; Millipore, Waters, Brussels, Belgium) was used. The mobile phase was methanol:water (adjusted to pH 3 with H_3PO_4):acetonitrile 40:40:20, (v/v/v). The flow rate was 1 ml/min, and 10 μl aliquots were injected. Propranolol (5 μg/ml) was used as the internal standard. UV detection was performed at 222 nm (11,12).

Statistical Analysis

The pulse times, lag times and ratio of cumulative quantities detected in the receptor compartment to the membrane area were compared by the Student t-test ($p < 0.05$).

The kinetics of drug permeation were compared by a two way analysis of variance (Scheffé F-test, $p < 0.05$).

RESULTS AND DISCUSSION

1. Pulses' Number

The influence of the electrical pulses' number on the transdermal passage of metoprolol was first studied. Therefore, a twin pulse composed of a first pulse of 300 HV and a second pulse of 100 LV was used. This twin pulse was applied 1, 5, 10, 15 or 20 times and every twin pulse was separated by 1 min.

The average pulse times of the first HV pulse and second LV pulse were 3.1 ± 0.1 ms and 621 ± 39 ms respectively. The resistance of the diffusion cell decreased from 17 ± 2 kΩ before the pulse to approximately 199 and 227 Ω respectively during the pulse; whereas skin resistance decreased from 16.5 ± 2 kΩ to approximately 74 and 72 Ω respectively.

As shown in fig. 1, application of the electric field pulse strongly enhanced the transdermal permeation of metoprolol as compared to the passive diffusion. It has already been reported that electroporation can achieve significantly ele-