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Skin electroporation for transdermal drug delivery: The influence of the order of different square wave electric pulses

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ABSTRACT

Electroporation can be used as an active enhancement method for intra- and transdermal drug delivery. Differences in response of skin to electric pulses depend on their amplitude, duration and number and have been a point of interest in the past. While protocols consisting of the same repetitive, mostly exponentially decaying pulses have been used before, this study is focused on comparing different combinations of square wave short high voltage (HV) and longer low voltage (LV) electroporation pulses. Our in vitro experimental results show that longer LV pulses significantly increase subsequent passive transport of calcein through dermatomed pig skin, while short HV pulses alone result in negligible calcein passive transdermal transport. Surprisingly, when the long LV pulses are preceded by short duration HV pulses, the total calcein transported is reduced significantly. This result is explained using a theoretical physics based model of individual local transport region (LTR) evolution during the applied LV pulse. The theoretical model shows that HV pulses alter the structure of the stratum corneum in such a way that when the LV pulses are applied, insufficient thermal energy is generated to initiate LTR expansion. Together, the experimental results and theoretical predictions show that the total pulse energy alone cannot account for total solute transport: that the order of the types of pulses administered must also be considered. Our findings open a direction for further improvement of the method using new protocols.

1. Introduction

In the past decades, the topic of intra- and transdermal drug delivery has become a field of biomedical research with rapid development. The success of this delivery route, however, depends on the ability of the drug to permeate the skin barrier in sufficient quantities to achieve its desired pharmacological effect. Modification of skin permeability to increase transdermal delivery has been achieved by passive (penetration enhancers, liposomes, nanoparticles, patch technology) and active methods of enhancement (electroporation, iontophoresis, microneedles, ultrasound, laser radiation). While passive methods are based on the formulation and chemical approaches, active employ physical force to overcome the skin barrier and/or provide a driving force on the drug (Williams, 2004; Zorec et al., 2013).

Electroporation is a well established and widely used method able to transiently create aqueous pores in phospholipid bilayers of cell membranes, using electric pulses of high voltage and short duration. It has also successfully been used to enhance skin permeability for molecules with different lipophilicity and size (small molecules, proteins, peptides and oligonucleotides), including biopharmaceuticals with molecular weight greater than 7 kDa (Denet et al., 2004; Denet and Préat, 2003). In addition, electroporation can also be used for applications where, after intradermal injection, the therapeutic molecules need to be inserted in the viable skin cells, or for gene transfection (André et al., 2008; Daugimont et al., 2010; Pavselj and Préat, 2005; Zibert et al., 2011). Transdermal transport after skin electroporation depends on the shape, amplitude, duration and the number of the electric pulses. When comparing different pulse protocols, pulse length and amplitude are the most influential factors in electroporation-based applications. Namely, to achieve the transmembrane voltage required for cell membrane permeabilization, a high enough pulse amplitude is essential. On the other hand, longer pulses are necessary to ensure higher

Abbreviations: SC, stratum corneum; LTR, local transport region; HV, high voltage; LV, low voltage.

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cumulative pulse energy, crucial for electrophoretic or resistive heating effects when needed.

It has been shown that molecular and ionic transport across the skin exposed to a number of high voltage pulses is highly localized in sites termed local transport regions (LTRs) (Pliquett, 1999; Pliquett and Gusbeth, 2004; Vanbever et al., 1999; Weaver et al., 1999). The size of the LTRs depends on cumulative pulse duration, while pulse amplitude dictates their density. The expert opinions in the field are in general agreement that there are different responses according to two primary pulsing regimes: short duration-high voltage (HV) pulses result in an altered stratum corneum (SC) that is perforated with micrometer-sized aqueous pathways while long duration low voltage (LV) pulses result in regions of increased permeability within the SC that are relatively large (up to hundreds of μm) and long lasting (Denet et al., 2004; Pliquett et al., 1996, 2005, 2008; Pliquett and Gusbeth, 2004; Vanbever et al., 1999). The structural alterations associated with the short pulse regime are accompanied by an increase in SC conductivity, which occurs within less than ~10 μs, when the voltage drop across the SC exceeds certain critical value (above ~50 V) (Denet et al., 2004; Pliquett et al., 2008; Pliquett and Gusbeth, 2004).

The response of the skin in the long low voltage (LV) pulse regime occurs at much longer timescales (up to hundreds of ms) and is associated with the development of "large" regions of altered SC. These long duration induced local transport regions (LTR) are formed in the sites of the so-called stratum corneum "defects" (Pliquett, 1999) that are expanded by resistive (Joule) heating. These large regions can originate as groupings of several of smaller pathways which expand to length scales of hundreds of μm. The large region of affected SC that results from this expansion is termed "local transport region" (LTR) because within this region the permeability is several orders of magnitude higher than in the surrounding unaltered SC. The development of the LTR is believed to be associated with resistive – Joule – heating which has been documented under certain experimental pulse conditions to cause localized temperature rises of over 60 °C (Pliquett et al., 1995, 2005, 2008; Pliquett and Gusbeth, 2000, 2004; Prausnitz, 1996; Vanbever et al., 1999).

The use of electric pulses on skin is not a new research topic. A number of studies have been performed, using both theoretical (Pavselj and Miklavcic, 2008; Pavšelj and Miklavčič, 2008, 2011) and experimental approach to explain the mechanisms involved. Since different parameters of electric pulses exert different effects on the stratum corneum, finding an optimal protocol has been a point of interest in the past. Earlier (and most) experiments were performed using repetitive exponentially decaying pulses (Pliquett et al., 1996, 2005; Pliquett and Gusbeth, 2000; Prausnitz et al., 1993; Regnier et al., 1999; Vanbever et al., 1998, 1999) of different amplitudes, durations and numbers, for two reasons: (i) the technology of capacitor-discharge devices delivering exponentially decaying pulses is much simpler than the technology behind square wave generators and (ii) the long low voltage tail of such pulses provides the electrophoretic part of the pulse, and is responsible for most of Joule heating, adding further to the success of transdermal delivery. On the other hand, to achieve better control of protocol parameters, square wave pulses are preferred (Denet et al., 2003; Denet and Préat, 2003; Dujardin et al., 2001, 2002). Again, different amplitudes, durations and number of square wave pulses have been used for transdermal delivery, albeit always with protocols composed of a number of one same type of pulses.

In our study we experiment with different combinations of square wave short high voltage (HV) and long low voltage (LV) pulses, focusing primarily on the order of pulses. The importance of the order of different types of pulses has been shown before in electroporation-based applications (Pavlin et al., 2010). However, to our knowledge, experiments that vary the sequence of different types of square wave pulses have not been done so far in studies that use electroporation to enhance transdermal delivery. The influence of pulsing protocol on calcein transdermal delivery associated with electroporation is investigated by using a combination of experimental observation and theoretical modeling. We begin with the experimental component of the study in which the total solute transported across the SC is measured for different pulse protocols in vitro. The second part is a theoretical description of the LTR development and its link to the observed experimental results, giving new insights into the mechanisms involved in skin electroporation. The authors have developed thermodynamic based models that directly relate the internal energy of the SC lipids to the degree of disorganization of the SC microstructure, linking it to molecular transport (Becker, 2011, 2012; Becker and Kuznetsov, 2007, 2008).

2. Materials and methods

2.1. In vitro experiments

2.1.1. General experimental setup

Vertical glass Franz diffusion cells were used to study molecular transport through excised and dermatomed pig skin. The temperature of the chamber was regulated at 37 °C by water circulation. A piece of porcine dermatomed skin was placed between two compartments with the stratum corneum facing the donor compartment. The area of skin available for diffusion was 0.785 cm^2. The receiver compartment (3.1 ml) was filled with PBS (pH 7.4, 150 mM), in order to maintain a constant pH and the osmolarity as well as to match ion concentration to that of human body. The donor compartment contained 1 ml of calcein solution (0.1 mM) in phosphate buffer (pH 6.5, 100 mM). For pulse delivery we used a unipolar square wave pulse generator Cliniporator (Igea, Italy). The pulses were delivered into the donor and the receiver compartment through 1 mm diameter platinum wire electrodes ending with a plate that is placed 0.2 cm away from the skin in donor compartment and 0.5 cm in the receiver compartment. As calcein is negatively charged, negative electrode was placed in donor and positive electrode in receiver compartment. When the skin is exposed to sufficiently intense electric pulses, its outer barrier, the stratum corneum undergoes a "breakdown" in its barrier properties due to local alteration of the microstructure of the stratum corneum lipids. The extent of the size of these alterations can vary by several orders of magnitude from the single pore (~10 nm) up to a larger local transport region (LTR) (~100 μm). Differences in response to electric pulses depend on pulse amplitude, duration and number. In order to study these differences, we experimented with both: short high voltage (HV) and long low voltage (LV) pulses, varying pulse number and order. Generally accepted postulation is that: (a) short, HV pulses (hundreds of μs) will result in the creation of small cell-level permeation pathways through the stratum corneum and (b) longer (hundreds of ms), low amplitude pulses will expand these pathways into the much larger LTRs, as well as provide some driving force for a charged solute before subsequent passive diffusion. The parameters of the HV pulses were: 500 V, 500 μs duration, 500 μs spacing between pulses (when applicable). Further, the parameters of LV pulses were: 45 V, 250 ms duration, 100 ms pulse spacing (when applicable). When HV pulses were followed by LV pulses, no delay between the HV and the LV part of the protocol was used. Pulse protocols and experimental conditions of the study are summarized in Table 1:

2.1.2. Chemicals and sample preparation

Calcein and sodium chloride were purchased from Sigma–Aldrich (St. Louis, MO, USA), while potassium chloride, di-sodium