Mathematical Biosciences 257 (2014) 60–68

Contents lists available at ScienceDirect

Mathematical Biosciences

journal homepage: www.elsevier.com/locate/mbs

Transdermal transport pathway creation: Electroporation pulse order

Sid Becker [a,*], Barbara Zorec [b], Damijan Miklavčič [b], Nataša Pavšelj [b]

[a] University of Canterbury, Christchurch, New Zealand
[b] University of Ljubljana, Ljubljana, Slovenia

ARTICLE INFO

Article history:
Available online 10 July 2014

Keywords:
Skin electroporation
Thermodynamic
Stratum corneum
Numerical
Experimental

ABSTRACT

In this study we consider the physics underlying electroporation which is administered to skin in order to radically increase transdermal drug delivery. The method involves the application of intense electric fields to alter the structure of the impermeable outer layer, the stratum corneum. A generally held view in the field of skin electroporation is that the skin's drop in resistance (to transport) is proportional to the total power of the pulses (which may be inferred by the number of pulses administered). Contrary to this belief, experiments conducted in this study show that the application of high voltage pulses prior to the application of low voltage pulses result in lower transport than when low voltage pulses alone are applied (when less total pulse power is administered). In order to reconcile these unexpected experimental results, a computational model is used to conduct an analysis which shows that the high density distribution of very small aqueous pathways through the stratum corneum associated with high voltage pulses is detrimental to the evolution of larger pathways that are associated with low voltage pulses.

1. Introduction

Advances in pharmacy and biotechnology have yielded a number of drugs that have shown promise in achieving good therapeutic results. Current trends show increased interest in the field of dermal and transdermal drug delivery. However, the skin's outer barrier, the *stratum corneum* (SC), offers the greatest resistance to molecular transport. Transdermal drug delivery methods focus on methods of overcoming this barrier function of the SC [1,2]. This study considers the physics underlying electroporation which involves the application of intense electric fields to alter the barrier's microstructure. By exposing the skin to electroporation pulses, the SC experiences a structural alteration that results in orders of magnitude increase in molecular diffusivity, electrophoretic mobility, and electrical conductivity.

The increase in the skin's permeability to transdermal delivery after skin electroporation depends on the electric pulse characteristics (i.e. amplitude, duration, number and frequency). The expert opinions in the field are in general agreement that there are different responses according to two primary pulsing regimes: (i) that short duration-high intensity pulses result in an altered SC that is perforated with nano- to micrometer-sized aqueous "pores", and (ii) that long duration medium intensity pulses result in regions of increased permeability within the SC that are relatively large (up to hundreds of μm) but that occur at a much lower density (number of pathways per SC surface area) than the high voltage (HV)-created pathways [3–15].

The structural alterations associated with the short pulse regime are probably related to the initiation of nanoscale aqueous defects into the SC's lipid microstructure. Experimental observations show that when the voltage drop across the SC exceeds some critical value (above ~30 V) the skin experiences a sudden increase in permeability to molecular transport of up to four orders of magnitude within less than ~10 μs [8]. The mechanism associated with alteration of the SC lipid structure during the HV pulses can be attributed to the interaction between the water dipole and the electric field. In molecular dynamics (MD) studies of single bilayer electroporation it has been shown that it is the polarity of water (and not the charge of the lipid head groups) that is responsible for the initiation of pores within the lipid structure [16,17]. The nanoscale aqueous pathways that are initiated within the first few μs of exposure to the electric field are a result of the interaction between the applied electric field and individual water molecules that are located at the surface of the SC and within any lipid structure defects throughout the interior of the SC.

The response of the skin in the long pulse regime occurs at much longer timescales (up to hundreds of ms) and is associated with the development of large regions of altered SC (up to hundreds of μm). The large region of affected SC that results from this expansion is termed the "Local Transport Region" (LTR) because within this region the permeability to molecular transport is

* Corresponding author. Tel.: +64 3 364 2987x7231.
E-mail address: sid.becker@canterbury.ac.nz (S. Becker).

http://dx.doi.org/10.1016/j.mbs.2014.07.001
0025-5564/

several orders of magnitude higher than in the surrounding unaltered SC. These LTRs are formed within the sites of the so-called *stratum corneum* "defects" [4] which have a higher electrical conductivity than the surrounding SC so that under the application of the low voltage (LV) electric pulses, the current density within these defects is high. 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 [3,6,9,10,18–21].

Based on published empirical data, the conclusion might be drawn that the skin's increase in permeability to molecular transport is proportional to the number of pulses administered (regardless of pulse type HV or LV). For example in the review by Denet [8] a compilation of data on the transdermal delivery of Fentanyl (net charge +1, MW 337 Da) shows that the total delivery increases with the number of pulses as well as with the pulse amplitude. This trend has been repeatedly shown in many experimental studies over the years. The conclusion from this can be drawn that more electric pulses will result in more transport regardless of the pulse characteristics. While this may be true for the transdermal delivery of solute of low molecular weight, an incorrect conclusion may be drawn that the increase in permeability (for all solutes) is proportional to the total power of the pulses. The authors of the present work believe that this is a false interpretation of the data and that one must consider the physics associated with pulse type in addition to the total power of the applied pulses.

Recently we have conducted experimental electroporation tests that provide results that are not really in agreement with the general view held by the field [22]. In fact the authors have shown that under certain pulsing protocols, the total transport of solute is *decreased* with the addition of more pulses. It is the motivation of this study to provide a preliminary investigation in which we consider the underlying physics responsible for LTR evolution and attempt to reconcile our experimental findings indicating that when long moderate voltage pulses are preceded by short intense pulses, the total transport is less compared to the case when long moderate pulses are administered alone.

2. Experimental results

The computational model presented is used to try to explain the empirical observations of *in vitro* experiments on dermatomed porcine skin. The experimental component of this paper follows an identical methodology to that reported previously [22]. The transport of calcein (molecular charge: 4−) was studied in vertical glass Franz diffusion cells (skin surface available for diffusion: 0.785 cm^2), thermo regulated at 37 °C by water circulation. A unipolar square wave pulse generator Cliniporator (Igea, Italy) was used for pulse delivery. The pulses were delivered into the donor and the receiver compartment through 1 mm diameter platinum wire electrodes placed 0.2 cm away from the skin in donor compartment and 0.5 cm in the receiver compartment. Different combinations of high voltage (HV) and low voltage (LV) pulses were used in the study (see Fig. 1). 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). Further, only about 30% of the voltage delivered with the pulse generator into the donor and the receiver solution was established across dermatomed skin. This skin voltage – U_{skin} – was measured during pulse delivery, as well as the electric current through the sample.

We monitored concentration of calcein in the receiver solution every hour for five hours of passive diffusion after pulse delivery. The concentration of calcein was measured with spectrofluorometer (Jasco, FP-6300). One way ANOVA was performed on all results as a statistical test (SigmaStat 3.1, Systat, USA). A 0.05 level of probability was taken as a level of significance. The results are expressed as the mean ± standard error of the mean (normality test passed in all instances). For further details about the experimental part of the study, we refer the reader to [22].

In Fig. 1 we present the experimentally obtained total concentration of calcein in the receiver solution, measured every hour for five hours of passive diffusion after pulse delivery. As expected, the control without an applied pulse to interfere with the barrier function of the SC results in negligible calcein concentration at five hours of passive diffusion after pulse delivery. On the other hand, only negligible rise in calcein delivery can be observed after 3 × HV pulse protocol, implying that the cell-size pathways through the SC created by short (500 μs duration) high voltage (HV) pulses are too small for significant enhancement of subsequent transdermal transport. A statistically significant improvement in the passive delivery of calcein following pulse delivery is accomplished only after longer LV pulses are added to the protocol. This is consistent with a very large increase in SC permeability that is anticipated in the creation of large LTRs which are associated with long LV pulses.

It is important to note that the system response to transport is very strongly tied to the exact conditions under which the experiments are conducted. Furthermore, the results depicted in Fig. 1 are specific to the delivery of calcein and should not be interpreted

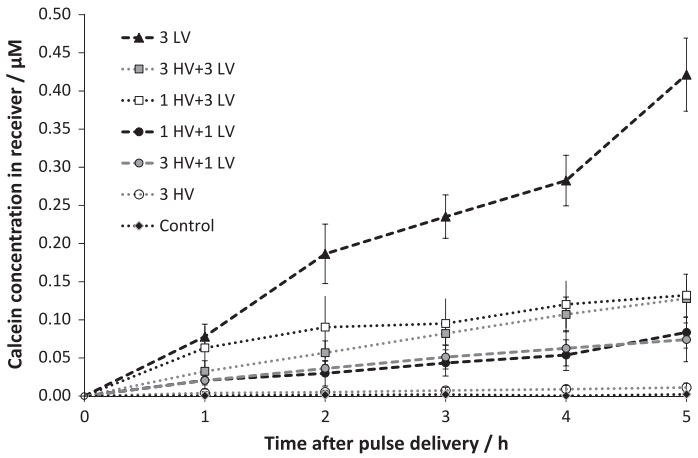

Fig. 1. Comparison of different combinations of short (500 μs) HV (high voltage) and longer (250 ms) LV (low voltage) pulses and their effect on calcein concentration in the receiver solution for 5 h of passive diffusion after pulse delivery. Pulses were delivered at time zero.