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Rapid Report

Transdermal insulin delivery using lipid enhanced electroporation

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Abstract

Transdermal insulin transport by electroporation was measured using porcine epidermis and fluorescein-labeled insulin. Previous studies have shown that anionic lipids can enhance the electroporative transport of molecules up to 10 kDa in size. It was also shown that it is the charge and not the type of the phospholipid head group that influences transdermal transport under electroporation. Moreover, phospholipids with saturated acyl chains enhance the transport of larger molecules more as compared to those with unsaturated chains. In the current study, based on those earlier findings, the effect of 1,2-dimyristoyl-3-phosphatidylserine (DMPS) on the transdermal transport of insulin by electroporation was examined. Porcine epidermis was used as a model for skin. Transport was measured using glass vertical diffusion apparatus in which the epidermis separated the donor and receiver compartments. Negative pulses were applied across the epidermis using platinum electrodes. Results show that when electroporation was carried out in the presence of DMPS, there was greater than 20-fold enhancement of insulin transport. Furthermore, while in the presence of the phospholipid, almost all the transported insulin was detected in the receiver compartment; in the absence of added lipids, only about half the insulin transported was in the receiver compartment and an almost equal amount of insulin remained in the epidermis. Fluorescence microscopy revealed that the insulin transport was mainly through the lipid multilayer regions that surround the corneocytes.

Keywords: Electroporation; Transdermal administration; Insulin; Stratum corneum; Skin; Porcine; Permeation; Liposome

1. Introduction

The *stratum corneum* (SC), the outermost layer of the skin, is the main barrier to transdermal transport. Electroporation has been used to deliver drugs and other molecules across the skin. The electrical resistance of the SC is between 5 and 25 $k\Omega/cm^2$ [1] and has an electrical breakdown potential around 75–100 V [2,3]. Electroporation creates new pathways (pores) resulting in transient permeabilization of the SC [4,5]. Small molecules like methylene blue [6] have been successfully transported into the epidermis/dermis regions by electroporation; similarly, the transport of metoprolol and fentanyl has been demonstrated using excised skin mounted between two compartments [7,8]. The molecular weight cutoff was determined, using FITC-dextrans, to be around 10 kDa [9,10]. Enhancement of transport by electroporation has been attempted using keratolytic molecules (sodium thiosulfate, urea, and heparin) with some success [11,12]. Recent studies show that the transports of molecules across skin were greatly enhanced by anionic lipid formulations [10]. The enhancement of transport occurs irrespective of the net charge for small (<1000 Da) molecules. In the case of larger molecules (4–10 kDa), this lipid enhancement of transport is only observed if the molecules are negatively charged. Insulin having a monomeric size of ~6 kDa and two net negative charges (at normal pH) is a suitable candidate drug for transdermal delivery. Transdermal insulin delivery has had very limited success. Previous workers have shown that delivery of monomeric insulin by iontophoresis is only possible with pretreatment of the skin by alcohol [13] or by depilatory cream [14]. According to the authors [13,14], the pretreatment with alcohol or the depilatory cream is likely to drastically reduce the barrier property of the skin thus enabling the transport of insulin. Moreover, the iontophoresis was carried for 2–3 h. The removal of the barrier property of the skin and the prolonged treatment time make such an insulin delivery system difficult and also in addition carries the risk of infection at the treatment site. When phospholipids are used to enhance the transport of molecules by electroporation, the skin

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recovers to its original resistive state, that is, its barrier property is restored in minutes thus reducing the risk of infection. Furthermore, the treatment time may also be reduced significantly (minutes) as compared to the 2–3 h required for iontophoresis [13,14]. The present study is directed at determining the effect of anionic phospholipids in enhancing the transdermal transport of insulin by electroporation.

2. Materials and methods

2.1. Lipid

1,2-Dimyristol-3-phosphatidylserine (DMPS) as chloroform solutions was obtained from Avanti Polar Lipids (Birmingham, AL). The lipids are stored at −80 °C until used. Lipid dispersions were prepared by first drying a measured amount of the lipid solution under a stream of nitrogen and placing the dry lipid under vacuum for an hour to remove any remaining solvent. The dry lipid was dispersed by vortexing in Tris buffer (10 mM Tris–HCl, 150 mM NaCl pH 8.0) at a final lipid concentration of 2 mg/ml.

2.2. Insulin

FITC-insulin (bovine) was obtained from Sigma-Aldrich Chemical (St. Louis, MO) as lyophilized powder and stored at −20 °C. Insulin solution was prepared at a concentration of 1 μg/ml in Tris buffer. The insulin solution was stored for a maximum of 1 week at 4 °C.

2.3. Porcine epidermis

Porcine full-thickness belly skin was obtained fresh from a local abattoir. Pieces of the skin were heated to 60 °C for 2 min and the epidermis was gently peeled off from the skin. The fresh epidermis was placed on glass microscope slides and kept dry at 4 °C until used. Before use, the epidermis was placed in a dish of distilled water and hydrated for an hour.

2.4. Electroporation

Insulin transport using electroporation in porcine epidermis was carried out using a glass vertical diffusion apparatus (Crown Glass, Somerville, NJ). A piece of epidermis was placed between two compartments, one serving as the donor and the other as the receiver compartment. Both compartments were filled with Tris buffer. The insulin (25 μg total) and the lipid dispersion (2 mg/ml) were added to the upper (donor) compartment. Platinum wire electrodes were placed close to the epidermis in both the donor and the receiver compartments. Electroporation was carried out using a pulse generator (Model 345, Velonex, Santa Clara, CA) delivering single or multiple unipolar square pulses of 100–105 V (1 ms pulse width at 1 Hz), with the negative electrode in the donor compartment.

2.5. Resistance measurements

Pre- and post-pulse epidermis and skin resistance was measured using the assembly described above. Continuous low voltage (700 mV, 1 kHz) bipolar square waves were applied to the epidermis using a Dynascan Model 3300, pulse generator (Chicago, IL). A load resistor (4.7 kΩ) was placed in series with the epidermis, and the voltage drop across the whole circuit (V_O) or just across the skin (V_S) was measured by a recording digital oscilloscope (Fluke 99 Scopemeter Series II, Holland). Skin resistance (in kilohms) was approximated from the formula

$$R_S = (V_S \times R_L)/(V_O - V_S),$$

where R_S = skin resistance and R_L = load resistor. A piece of epidermis was only used if the pre-treatment resistance equaled 6 kΩ or more.

2.6. Fluorescence measurement

The amount of FITC-insulin transported across the epidermis to the receiver compartment was measured by removing the entire content of the receiver and drying to 2.5 ml. The amount of insulin that was retained in the epidermis was determined by dissolving the epidermis in Solvable™. Fluorescence intensities were measured in a SLM 8000 Spectrofluorimeter (SLM Instruments, Urbana, IL). Excitation and emission wavelengths were 491 and 517 nm, respectively. The amount of insulin transported was determined from the measured fluorescence intensity using a calibration curve prepared using known amount of FITC-insulin.

2.7. Fluorescence microscopy

The epidermis was viewed using an Olympus IMT-2 microscope with a 20 × objective and fluorescence images recorded with the help of a SPOT digital camera. No processing was done to enhance the recorded images.

3. Results and discussion

3.1. Insulin transport

The enhancing effect of DMPS on the transport of insulin was examined by measuring the transport in the presence and the absence of DMPS (2 mg/ml). The epidermis was pulsed for a total of 10 min. The plot of the total insulin transported to the receiver compartment in the presence and the absence of DMPS is shown in Fig. 1. When the epidermis is electroporated for 10 min in the

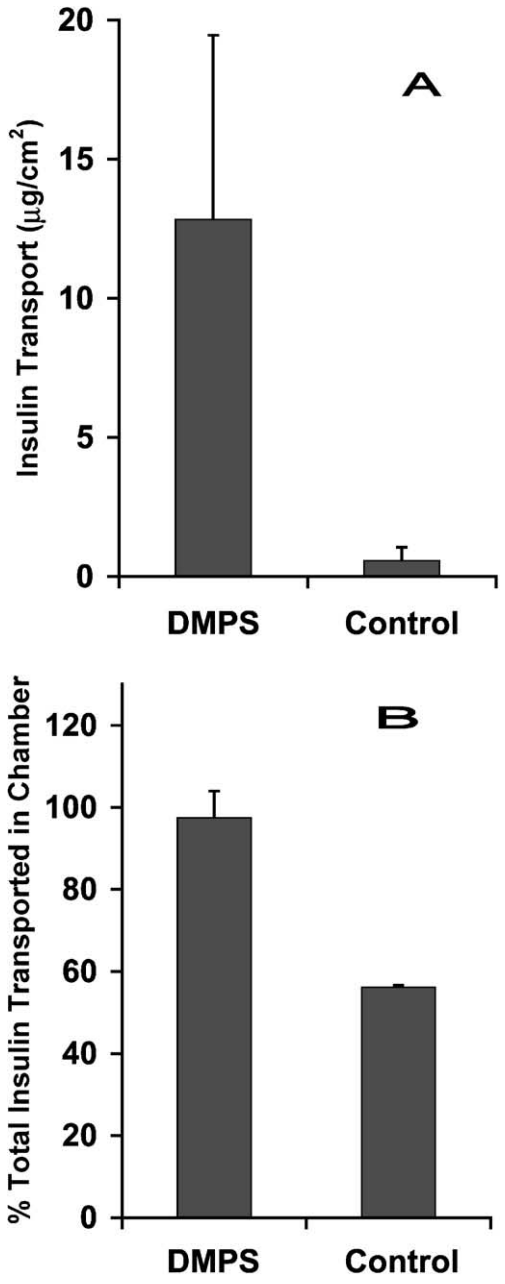

Fig. 1. Transdermal insulin transport under electroporation with and without added DMPS (2 mg/ml) using 100 V negative pulse (at 1 Hz and 1 ms pulse width) (A) for 10 min, and the percentage of the total insulin transported that is in the compartment under those conditions (B). The rest of the insulin remained in the epidermis.

absence of DMPS, with insulin in the donor compartment, there is very little transport of the drug to the receiver compartment (<0.6 μg/cm^2 total) (Fig. 1A). When DMPS was added together with the insulin, the total insulin transport in the receiver compartment was around ~ 13 μg/cm^2 (Fig. 1A). The enhancement in insulin transport is thus around 20-fold. The enhancement in transport due to added mixed phospholipids (containing an anionic phospholipid DOPG and DOPC at1:1 molar ratio) observed for different molecular-weight Dextrans shows a size dependence and the enhancement is $\sim$ 15-fold for a 4-kDa Dextran [8]. The anionic lipid-mediated transport enhancement can be as high as 80-fold (4 kDa Dextrans) with added DMPS [15]. Almost all of the insulin transported by electroporation in the presence of DMPS is found in the receiver compartment, while only about 50% of it is in the receiver compartment if the lipid was not added during electroporation (Fig. 1B). The other half of the insulin transported was retained in the epidermis. However, because of the large amount of insulin transport in the presence of added lipid, the actual amount of insulin in the epidermis is greater in the presence of DMPS.

3.2. Fluorescence imaging

The FITC-insulin retained in the epidermis after electroporation can be observed by fluorescence microscopy (Fig.

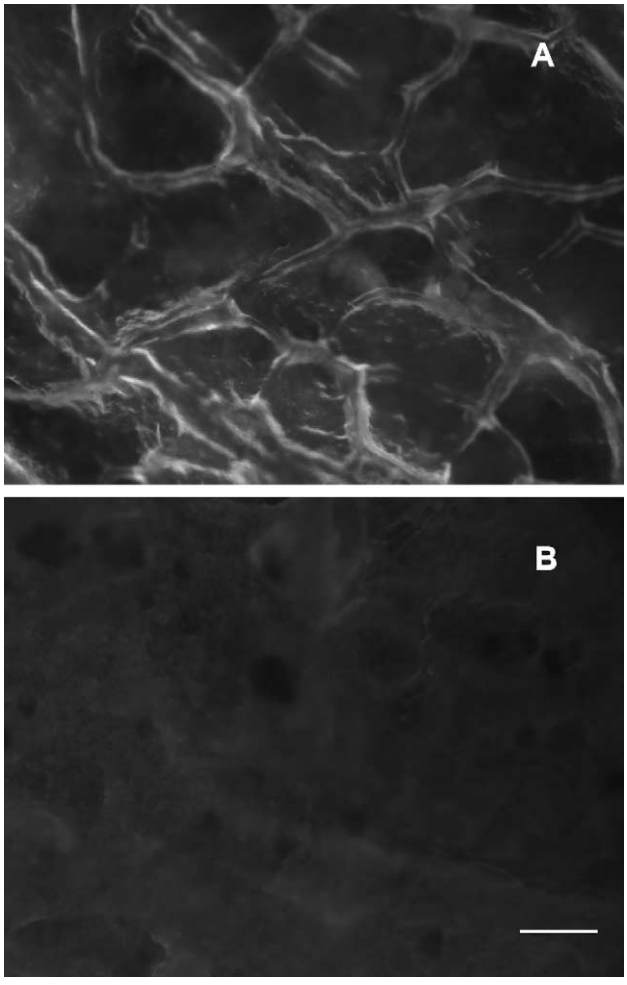

Fig. 2. Fluorescence photomicrographs of porcine epidermis after transport of FITC-labeled insulin under electroporation with (A) and without (B) added DMPS. The illumination and the exposure were the same in both photomicrographs. The bright fluorescence from the FITC label on the insulin is seen around the corneocytes. Scale bar represents 50 μm.

2A,B). Most of the fluorescence is seen in the lipid multilayers around the corneocytes indicating that the transport route is primarily through these regions of the epidermis. The images obtained from epidermis in which the electroporation was conducted in the presence and the absence of DMPS show a similar distribution of the FITC-insulin. This supports our earlier observation that the localized transport regions are primarily in the lipid regions of the epidermis.

Anionic phospholipids have recently been shown to enhance the transdermal transport of molecules during electroporation [10]. The enhancement is dependent upon the charge and the size of the delivered molecule. The anionic lipid-mediated enhancement is small if the transported molecule is small and negatively charged, and the enhancement is large for positively or uncharged small molecules [10]. However, in the case of larger molecules (MW $\simeq$ 4 kDa), the enhancement is greater for negatively charged molecules. There is very little, or no enhancement due to added anionic lipids observed for large uncharged or positively charged molecules [10] under electroporation. Anionic phospholipids with saturated acyl chains were found to enhance transport much more than the same phospholipid with unsaturated acyl chains [15]. DMPS (1 mg/ml) was found to enhance the transport of 4-kDa and 9-kDa Dextrans by 80- and 90-fold, respectively [15]. The enhancement found here for FITC-insulin is around 20-fold, which is less than that found for 9-kDa dextrans. The monomeric molecular weight of insulin is around 6 kDa and if it had the same charge/mass ratio as that for the Dextrans used in the earlier work, one would expect the DMPS-induced enhancement would have been greater. There are two possible reasons for the lower enhancement of transport for insulin in the presence of added anionic lipids. The first is that insulin carries only two net negative charges per molecule. The charge on the Dextrans used is greater. (The exact charge to mass ratio of the Dextrans used is not known. The Dextrans are labeled with between 0.003 to 0.02 mol FITC per mole glucose.) The second possibility is that while the monomeric size of insulin is 6 kDa, it is known to form dimers and hexamers (in presence of zinc)[16,17]. The resultant size of insulin would thus increase to 12 or 36 kDa dependent upon the presence of zinc. Since no effort was made to ensure that the product used was zinc-free, it is expected that a large majority of the insulin would be in a hexameric state. An enhancement of 20-fold in transport by electroporation seen with added DMPS is thus in the range expected from the earlier studies with dextrans. The results indicate that if monomeric insulin were used, it would be possible to deliver very much greater amounts of insulin by transdermal electroporation. Therapeutic levels of insulin delivery are thus very likely using phospholipid-enhanced electroporation.

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