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Synergistic effect of anionic lipid enhancer and electroosmosis for transcutaneous delivery of insulin

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Abstract

A lipid formulation consisting of 1,2-dimyristoyl-sn3-phosphatidylserine (DMPS) in a 0.2% sodium dodecylsulfate (SDS) solution was tested as an in vivo enhancer for the transcutaneous delivery of insulin. The formulation when applied to for 15 min was found to permeabilize porcine epidermis and prolong the permeable state as evidenced by electric resistance measurement. The formulation enhanced the transport of insulin through the epidermis by 40- to 100-fold, as compared to epidermis that was treated with SDS or DMPS alone. Application of electroosmosis across the formulation-treated epidermis enhanced the transport of insulin by an additional 10-fold. Pharmacokinetic studies were carried out in Sprague-Dawley rats. Transcutaneous delivery of insulin with formulation treatment and electroosmosis increased the plasma level of insulin by ~10-fold over delivery by formulation treatment alone. With the above protocol plasma insulin concentration remained relatively constant for up to 4 h. The synergistic application of anionic lipid formulation and electroosmosis offers a promising non-invasive technique to deliver insulin transcutaneously.

Keywords: Anionic lipid; Insulin; Porcine epidermis; Rat skin; Transcutaneous delivery; Electroosmosis; Pharmacokinetics

1. Introduction

Insulin is a 51 amino acid peptide generally administered parenterally for treating diabetes mellitus (Chien, 1996). Injections generally are not ideal methods for the administration of drugs, especially for chronic conditions. There have been many attempts to deliver insulin by non-invasive methods. These include oral, colonic, rectal, ocular, buccal, pulmonary, uterine and transdermal routes (Hoffman and Ziv, 1997). Oral delivery of insulin is affected due to degradation by the proteolytic enzymes in the G.I.T. (Aboubakar et al., 1999). Mucosal delivery is not effective because of its poor absorption by mucosae of nasal, rectal, pulmonary and ocular route (Damge et al., 1997). Transdermal delivery has many advantages over other delivery methods, the most important being its convenience. The skin also serves as a reservoir for controlled release. Transdermal delivery of insulin is limited by the low permeability of the stratum corneum (SC), which consists of flat, enucleated cells filled with keratin fiber surrounded by lipid bilayers. Several approaches such as iontophoresis and sonophoresis have been taken to bypass the skin barrier properties (Tomohira et al., 1997; Kankkannan et al., 1999; Langkjaer et al., 1998; Smith et al., 2003; Boucaud et al., 2002). However, the flux for even monomeric insulin resulting from iontophoresis was low and variable (Langkjaer et al., 1998), because iontophoresis works best only for small, charged molecules that pass through the appendage routes of the skin. The time required to achieve therapeutically relevant concentrations was relatively long. Pillai et al. (2004) applied iontophoresis onto chemically treated or permeabilized skin for improved transport of insulin. Unless significant passages for solutes are created and maintained in the SC to allow larger molecules to transit, transdermal delivery of insulin will remain a challenge.

We reported the phenomenon of prolonged permeabilization of mammalian epidermis on incorporation of an anionic lipid, dimyristoyl-phosphatidylserine (DMPS), in the SC lipid domain with the aid of electrical pulses (Sen et al., 2002a, 2002b). The perturbation of SC lipids by joule heating associated with applied electrical pulses was demonstrated recently Martin et al., 2002; Pliquett et al., 2005). It is likely that the exogenous lipids

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like DMPS get incorporated in the SC lipid lamellae due to the phase transition of SC lipid brought about by the joule heating and the incorporated DMPS retard the reformation of the SC barrier. In this work, we tried to incorporate DMPS in the SC lipid lamellae without the application of electrical pulses but with the aid of external heating to 40 °C which corresponds to the first transition temperature of the SC lipids (Golden et al., 1987) and by using a small amount of SDS as a vehicle. We found that treating porcine epidermis with a lipid formulation consisting of DMPS (in 0.2% SDS) at 40 °C for 15 min renders the skin permeable for prolonged duration. The treatment is referred to as "formulation treated" in this paper. We further tested for synergy between formulation treatment and iontophoresis in the delivery of insulin across the skin. Our mechanistic studies have revealed that at physiological pH, insulin is delivered mainly by electroosmosis and only minimally by electromigration (Murthy et al., 2006). Therefore, we use the term "electroosmosis" in all the experiments involving application of dc (the anode was placed in the donor and cathode in the receiver compartment) in this study.

2. Materials and methods

2.1. Chemicals

Fluorescein isothiocyanate (FITC) labeled insulin and other chemicals used were from Sigma–Aldrich and Fisher Chemical Company (St. Louis, MO). FITC-insulin was dialyzed for 48 h using 3500 Da dialysis membranes (Spectrapor®) to remove any free dye. Centrifree filtered ^{125}I-labeled porcine insulin was purchased from Amersham Biosciences (Piscataway, NJ). Dulbecco's phosphate buffered saline (PBS pH 7.2, free of calcium) was purchased from Gibco (Grand Island, NY).

Dimyristoyl-phosphatidylserine (DMPS) was purchased from Avanti Polar Lipids (Birmingham, AL). Lipid dispersions were prepared by drying the lipids from chloroform solutions, and then vortexing in PBS buffer at a final lipid concentration of 2 mg/ml. Sodium dodecylsulfate (SDS) was added to the suspension at 0.2% (w/w). This is referred hereon as the "formulation".

2.2. Skin for in vitro studies and animals for in vivo studies

Porcine belly skin was excised from freshly euthanized experimental animals. Pieces of the skin wrapped in aluminum foil were heated to 60 °C for 2 min in a water bath and the epidermis was gently peeled off the skin. The fresh epidermis was placed on glass microscope slides and kept dry at 4 °C until used. Prior to use, the epidermis was hydrated with normal saline (0.9% (w/v) sodium chloride) for 1 h. The procedure follows that used by Chizmadzhev et al. (1998) for preserving human epidermis.

Sprague-Dawley rats were purchased from Jackson Laboratory (Bar Harbor, ME). Animals weighing about 250–300 g were used in the study. Animals were provided free access to food and water and were allowed at least 5 days to recover after transportation. All animals were kept in the Institute Animal Facility before and during experiment.

2.3. In vitro experimental setup

Franz type vertical diffusion apparatus (Crown Glass Company Inc., Somerville, NJ) was used for all resistance and transport measurements across porcine epidermis. The temperature of the chamber was regulated by water circulation to 40 °C. A piece of porcine epidermis was placed between two compartments of the diffusion apparatus, one serving as the donor and the other as the receiver compartment. The area of epidermis available for diffusion was 0.64 cm^2. The volume of donor and receiver compartment was 0.5 and 5 ml, respectively. Ag/AgCl electrodes of 5 mm diameter (InVivo Metric, Healdsburg, CA) were placed 2 mm away from the skin in both the donor and the receiver compartments.

2.4. Electrical measurements

The resistance of the epidermis was measured by placing a load resistor R_L (4.7 kΩ) in series with the epidermis. The voltage drops across the whole circuit (V_O) and across the epidermis (V_S) were measured using a recording digital oscilloscope (Fluke 99 Scopemeter series II, Eindhoven, The Netherlands). Epidermis resistance (in kΩ) was approximated from the formula:

$$R_S = \frac{V_S R_L}{V_O - V_S}$$

where R_S is the epidermis resistance and R_L is the load resistor in kΩ. A piece of porcine epidermis was used only if it had a resistance greater than 50 kΩ/cm^2.

During electroosmosis, the electrodes were connected to a regulated dc constant current supply with the anode in the donor compartment and the cathode in the receiver compartment.

Four sets of experiments were carried out involving the following treatment. Porcine epidermis was maintained at 40 °C with the buffer for 10–15 min until the resistance became constant ($R_S = R_O$). Then the epidermis was treated for 15 min with one of the following: (1) buffer (control/untreated), (2) SDS (0.2% (w/w)), (3) DMPS (2 mg/ml) alone and (4) the formulation. Time-dependent changes in the electrical resistance $R_S(t)$ of the epidermis with the different treatments were measured and used to calculate the relative resistance $R_S(t)/R_O$.

In skin recovery studies, the epidermis was transferred to a new diffusion cell maintained at 37 °C and the electrical resistance of the epidermis was measured over a time period. The relative resistance, the ratio of resistance at different times at 37 °C $R_S(t)$ to the initial resistance of the epidermis measured at 40 °C before any treatment (R_O) was calculated.

2.5. In vitro insulin transport

The receiver compartment of the diffusion chamber was filled with PBS and FITC-insulin solution (2 mg/ml solution in PBS) was added to the donor chamber. For electroosmosis, a constant dc (0.5 mA/cm^2) was applied across the epidermis. The amount of insulin transported across the epidermis was determined from the measured fluorescence intensity of the FITC-insulin in the receiver compartment. The insulin solutions present in both