Passive and Iontophoretic Transport Enhancement of Insulin Through Porcine Epidermis by Depilatories: Permeability and Fourier Transform Infrared Spectroscopy Studies

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ABSTRACT

The effect of thioglycolate-based depilatory lotions was studied on the in vitro passive and iontophoretic permeability of insulin through porcine epidermis and biophysical changes in the stratum corneum (SC) lipids and proteins. The porcine epidermis and Franz diffusion cells modified for iontophoresis were used for the in vitro transport studies. Cathodal iontophoresis was performed at 0.2 mA/cm² current density. Resistance of the control- and depilatory-lotion–treated epidermis was determined according to Ohm’s law. Biophysical changes were studied on porcine SC before (control) and after treatment with the depilatory lotions using Fourier transform infrared (FT-IR) spectroscopy. Asymmetric (~2915 cm⁻¹) and symmetric (~2848 cm⁻¹) Carbon-Hydrogen (C-H) stretching absorbances were studied to estimate the extent of lipid extraction. Fourier self-deconvolution and second derivative procedures were applied to amide I band (1700-1600 cm⁻¹) in order to estimate quantitatively the changes in the secondary structure of the SC protein. The passive permeability of insulin was significantly (P < .05) increased through depilatory-lotion–treated (ie, Better Off, Marzena, and Sally Hansen) epidermis in comparison to control. Iontophoresis significantly enhanced (P < .05) the permeability of insulin through depilatory-pretreated epidermis in comparison with the control epidermis. Further, we were able to achieve the desired flux of insulin (5.25 U/cm²/d) through Better Off–treated epidermis using 0.2 mA/cm² current density and 100 U/mL donor concentration of insulin. The SC treated with depilatory lotions showed a decrease in peak areas of C-H stretching absorbances in comparison with untreated SC. Depilatory lotion treatment also decreased (P < .05) the epidermal resistance in comparison with the control epidermis. The decrease in the α-helix conformation and the increase in the random and turn structures were observed in the SC proteins due to depilatory lotion treatment. The changes in the secondary structure of proteins and lipid extraction from the SC are suggested as the cause of the decrease in the epidermal resistance and the increase in the passive and iontophoretic permeability of insulin through depilatory-pretreated epidermis in comparison with the control epidermis.

KEYWORDS: transdermal, insulin, depilatory lotion, iontophoresis, epidermal resistance

INTRODUCTION

Different techniques have been used to increase the transdermal transport of insulin. Stripping of the stratum corneum (SC)¹ and use of penetration enhancers² have been reported to increase the transdermal transport of insulin. Delipidization of mouse skin by gentle wiping with absolute alcohol increased the iontophoretic flux of insulin.³ Studies have suggested that depilatory lotions alter skin permeability by changing the skin barrier function.⁴,⁵ A depilatory cream significantly increased the skin absorption of testosterone.⁶ Calcium thioglycolate, when used as a penetration enhancer, led to a 40-fold increase in the plasma concentration of theophylline.⁷

Dry SC is composed of (by weight) approximately 75% protein, 25% lipid, and a small percentage of low molecular weight materials. Skin penetration enhancers can either alter the lipid structure or interact with intercellular SC lipid, or simultaneously exert both actions, resulting in improved drug permeation. The role of the
lipids in the barrier function of the SC is well established, and techniques to reduce their role are often used in transport studies. Proteins, being the major component of the SC, draw attention for investigations. Denaturation of keratin in the SC has been suggested as a mechanism for permeability enhancement of SC. SC contains 2 fibrous proteins, 1 with an α-helical conformation and the other with a cross-β-structure, the latter being the minor component. Protein conformational changes were noticed when porcine SC was pretreated with skin penetration enhancer–azone/propylene glycol. Five percent azone in propylene glycol as a penetration enhancer increased the iontophoretic flux of insulin by a factor of 2.75 as compared with iontophoresis alone. Therefore, the SC proteins might play an important role in the transdermal transport enhancement of insulin.

Electrical resistance of the skin is an average measure of the difficulty that the charge carriers have in traversing the skin. Changes in electrical properties also indicate the changes in membrane properties of the skin. Fourier transform infrared (FT-IR) spectroscopy allows the simultaneous investigation of the lipid and protein components of a biological membrane. This technique can be employed to study the extent of lipid extraction from porcine SC. Decreases in the absorbances of C-H stretching peaks have been linked to the SC lipids extracted from porcine SC. Decreases in the absorbances of C-H stretching peaks have been linked to the SC lipids extracted from porcine SC. Decreases in absorbance of C-H stretching peaks have been linked to the SC lipids extracted from porcine SC.

Fourier transform infrared (FT-IR) spectroscopy is an appropriate separation technique. The epidermis was prepared from porcine ears by heat separation technique. The whole skin was soaked in normal saline (0.9% wt/vol sodium chloride) for 45 seconds, followed by careful removal of the epidermis. The epidermis was washed with deionized water and used in the in vitro percutaneous absorption studies. SC samples from the epidermis were prepared using the trypsin digestion method.

The application of a depilatory lotion prior to the transport study was found to enhance the passive and iontophoretic transdermal delivery of insulin. However, these studies did not elaborate the mechanism of transport enhancement. It is important to recognize the changes in the SC by the depilatory lotion treatment in order to understand the mechanism of transport enhancement of insulin.

The overall objective of our research is to achieve the transdermal transport of therapeutic doses of insulin required for the treatment of diabetes. This study, for the first time, would provide the biophysical basis of understanding the mechanism of transport enhancement of insulin through depilatory-lotion-treated porcine epidermis. This study investigated the effects of pretreatment of the epidermis with depilatory lotions on the epidermal resistance and in vitro passive and iontophoretic transepidermal transport of insulin. Changes in protein secondary structures and the extent of lipid extraction of the SC by depilatory lotions were studied by FT-IR spectroscopy.

**Materials and Methods**

**Materials**

Thioglycolate-based depilatory lotions used were Better Off (Personal Care Group Inc, Montvale, NJ), Marzena (Marzena Bodycare Products Inc, Badger, CA), and Sally Hansen (Del Laboratories Inc, Farmingdale, NY). [125I] Human recombinant insulin (specific activity: 2000 Ci/mmol) was purchased from Amersham Pharmacia Biotech Inc (Piscataway, NJ). Lispro insulin (Humalog) was obtained from Eli Lilly and Company (Indianapolis, IN). All chemicals and reagents used were of analytical grade. Deionized water (Resistivity ≥ 18 MΩ-cm) was used to prepare all solutions and buffers.

**Preparation of Epidermis and SC**

The epidermis was prepared from porcine ears by heat separation technique. The whole skin was soaked in water at 60°C for 45 seconds, followed by careful removal of the epidermis. The epidermis was washed with water and used in the in vitro percutaneous absorption studies. SC samples from the epidermis were prepared using the trypsin digestion method.

**In Vitro Transport**

Depilatory lotion (100 mg/cm²), sufficient to spread over the sample, was used to treat the epidermis for 10 minutes and then washed with deionized water. Franz diffusion cells, modified for iontophoresis, were used in the in vitro transport studies. The treated or untreated (control) epidermis was sandwiched between the cells with the SC facing the donor compartment. The maximum capacity of the donor and receiver compartment was 1 mL and 5 mL, respectively. The effective diffusional area was 0.785 cm². The donor compartment contained 1 mL of insulin solution (0.2 µCi of insulin in 0.9% wt/vol sodium chloride [normal saline]), and the receiver compartment was filled with 5 mL of normal saline. The donor concentration of insulin used