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Effect of different enhancers on the transdermal permeation of insulin analog

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ABSTRACT

Using chemical penetration enhancers (CPEs), transdermal drug delivery (TDD) offers an alternative route for insulin administration, wherein the CPEs reversibly reduce the barrier resistance of the skin. However, there is a lack of sufficient information concerning the effect of CPE chemical structure on insulin permeation. To address this limitation, we examined the effect of CPE functional groups on the permeation of insulin. A virtual design algorithm that incorporates quantitative structure–property relationship (QSPR) models for predicting the CPE properties was used to identify 43 potential CPEs. This set of CPEs was prescreened using a resistance technique, and the 22 best CPEs were selected. Next, standard permeation experiments in Franz cells were performed to quantify insulin permeation.

Our results indicate that *specific* functional groups are not directly responsible for enhanced insulin permeation. Rather, permeation enhancement is produced by molecules that exhibit positive $\log K_{ow}$ values and possess at least one hydrogen donor or acceptor. Toluene was the only exception among the 22 potential CPEs considered. In addition, toxicity analyses of the 22 CPEs were performed. A total of eight CPEs were both highly enhancing (permeability coefficient at least four times the control value) and non-toxic, five of which are new discoveries.

1. Introduction

Non-traditional methods for insulin delivery like insulin pumps, inhalers and pens are gaining importance due to their obvious advantages over traditional delivery methods (Patni et al., 2006). Another promising non-traditional alternative is the delivery of insulin through skin using transdermal patches. Transdermal drug delivery (TDD) can minimize problems commonly associated with traditional delivery methods such as, painful administration, patient compliance, liver metabolism, and sustained and controlled delivery (Chong and Fung, 1989). As such, TDD has gained wide acceptance in many therapeutic applications for a wide variety of drugs like nicotine (Nair et al., 1997) and estradiol (Goodman and Barry, 1988).

However, delivery of protein molecules like insulin has proven to be difficult due to the large molecular size (>3000 Da) and hydrophilic nature of these molecules to which the human skin provides a very efficient transport barrier (Monteiro-Riviere, 1991, 1996). Several physical and chemical methods have been developed to improve the permeation of insulin through human skin (Pillai and Panchagnula, 2003a; Rastogi and Singh, 2003; Pan et al., 2002; Smith et al., 2003). By far, the most efficient method in enhancing insulin permeation through skin is iontophoresis (Pillai and Panchagnula, 2003a); however, the economic viability and ease of applicability of chemical approaches, such as the use of chemical penetration enhancers (CPEs), makes them an attractive alternative.

Very few studies involving the use of CPEs for transdermal insulin delivery exist in the literature (Rastogi and Singh, 2003; Pillai et al., 2004). Further, in these limited studies, traditional CPEs involving either fatty acids or fatty alcohols are employed in tandem with iontophoresis. As such, CPEs in these applications are not mixed in a solution along with insulin. Instead, the skin is pretreated with the CPE solution for about 2 h, and then washed off before placing the insulin solution on the skin. This is inconvenient from the standpoint of the applicability of an insulin patch designed for sustained TDD. More importantly, these CPEs are limited in their ability to increase the permeation of insulin through the skin significantly and/or exhibit toxic effects to the skin. To address these shortcomings, a large number of CPEs (43 CPEs) containing different functional groups were selected to examine the effect of CPE structural variation on insulin permeation. The CPEs were not used for pre-treating the skin; rather, they were mixed with the insulin solution and allowed to remain in contact with the skin.

The 43 CPEs were identified with our virtual design algorithm which combines genetic algorithms (GAs) and quantitative structure–property relationship (QSPR) models for important CPE properties (skin permeation, octanol–water partition coefficient, melting point, aqueous solubility, and skin sensitization).

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Specifically, the development of the virtual design algorithm included (a) integration of non-linear, theory-based structure–property relationship (SPR) property models and genetic algorithms (GAs) to develop a reliable virtual screening algorithm for generation of potential CPEs and (b) validation of the virtual screening predictions (using melatonin as an example drug and pig skin as the model for human skin) by performing carefully designed experiments for CPE toxicity and drug permeation in the presence of potential CPEs identified as non-toxic.

Details concerning the algorithms and models can be found elsewhere (Godavarthy et al., 2009). Based on functional group attachments, this set of CPEs was classified as: fatty acids, fatty ketones, fatty alcohols, fatty aldehydes, fatty amines, pyridines/pyrrolidones/pyrrolidinones, alkanes/alkenes/alkynes, halogenoalkanes/alkenes and other miscellaneous CPEs not belonging to any of the previous groups. Only molecules that had positive logarithmic octanol–water partition coefficient ($\log K_{ow}$) values were selected because these molecules are more likely to exhibit desired skin transport properties. As such, only molecules that increase the permeation of drugs through the "pull" mechanism proposed by Liron and Cohen (1984) and Kadir et al. (1987) have been investigated. The permeation effect of this mechanism results from the interaction of a suitable CPE with skin which alters the dermal properties allowing increased permeation of a drug of interest. Significant properties of the CPEs calculated using ChemAxon (2007) software are tabulated in Table 1.

The traditional permeation experimental apparatus using Franz diffusion cells provides a reliable *in vitro* technique for estimating the permeation of insulin through the skin; however, it is time and labor-intensive. Therefore, a two-step approach for identifying potential CPEs is employed. First, only those CPEs that decrease the skin resistance beyond a threshold value were selected using a resistance technique, which is justified based on our previous experience and similar studies reported in the literature (Karande et al., 2004, 2006; Rachakonda et al., 2008). Second, traditional permeation experiments were performed to calculate accurately the permeability of insulin.

Another important issue associated with the use of CPEs is their toxicity. In fact, toxicity is often the major limiting factor in identifying potential CPEs (Karande et al., 2004). To assess the suitability of the CPEs investigated in the present study, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-formazan assay was employed to evaluate cell viability in the presence of a CPE of interest, and this constituted a preliminary screening mechanism to eliminate CPEs that were toxic at the cellular level. Additionally, histology studies on the intact porcine skin were performed to examine the effects of CPEs on skin.

2. Materials and methods

2.1. Materials

All of the enhancers studied were obtained from Sigma–Aldrich Chemical Company (St Louise, MO, USA), except for 1-methyl-2-pyrrolidone, which was obtained from ConocoPhillips (Bartlesville, OK, USA). Lispro, a short acting analog of human insulin, was purchased from Eli Lilly Company (Indianapolis, IN, USA). Lispro was selected because of its higher dermal absorption rate than normal insulin, which makes it more suitable for transdermal delivery. Henceforth, in the present work, insulin refers to the Lispro analog. Ethanol USP was obtained from Aaper Alcohol and Chemical Co. (Shelbyville, KY, USA). High performance liquid chromatography (HPLC) grade acetonitrile was purchased from Fischer Scientific (Atlanta, GA, USA).

2.2. Skin preparation

Fresh porcine abdominal skin was obtained from Ralphs Packing Co. (Perkins, OK, USA) and Bob McKinney & Sons (Cushing, OK, USA). The skin was cleaned with cold running water to remove dried blood and dirt, and hair was clipped using an electric clipper. The adipose tissue and subcutaneous muscle layers were surgically excised. The skin was then wrapped in aluminium foil and stored at −20 °C until needed. The skin was stored for a maximum of 4 weeks before being used for experimentation. Representative samples were assessed histologically to assess the quality of the skin preparation, as described in Section 2.6.

2.3. Pre-screening of enhancers based on changes in skin resistance

2.3.1. Preparation of solution

Phosphate buffered saline (PBS, pH–7.4, phosphate and sodium chloride concentrations of 0.001 and 0.137 M, respectively) was used to prepare CPE solutions on the day of the experiment. First, 0.5 mL of ethanol was mixed with 0.5 mL of PBS and the test CPEs were added to each of these solutions to give a final CPE concentration of 5% (w/v).

2.3.2. Experimentation

The enhancers were pre-screened based on their skin resistance reduction, which was measured using a resistance chamber that was built in-house. A schematic of the chamber and specific details of this technique, including the current type, the current intensity and the instrumentation are described elsewhere (Rachakonda et al., 2008). Briefly, the resistance chamber consists of two half-inch thick Teflon plates fixed to a Teflon Petri dish. Five holes with a diameter of 0.79 cm were drilled into each Teflon plate. The holes in the top plate serve as donor chambers, and the holes in the bottom plate serve as receiver chambers, as in Franz diffusion cells. Porcine skin was placed between the receiver and donor plates with the stratum corneum facing the donor wells, and the two plates were clamped together tightly. The Petri dish was filled with PBS such that the receiver chambers were completely filled with PBS, which was assured by checking the skin resistance; presence of air pockets between the skin and the receiver chambers showed very high resistance values since air has low conductivity. Resistance readings were taken using a common electrode placed beneath the receiver plate and the other placed sequentially into each donor well, which were filled with just the CPE and no insulin.

All potential enhancers were tested at a concentration of 5% (wt/v) in 1:1 PBS and ethanol solution with the receiver chambers maintained at 37 ± 1 °C. Resistance measurements were taken every hour for up to 6 h and the CPE solution was in contact with the skin for this entire period. Rachakonda et al. (2008) have shown that the efficacy of a CPE can be established by measuring the drop in the skin resistance during a 6-h contact period between the CPE and the skin. Measurements beyond this time period contribute no additional information regarding CPE efficacy. The resistance reduction factor (RF), which is defined as the ratio of the initial resistance value (R) at time 0 to the resistance value of the sample obtained at time t (6 h), was calculated as given by:

$$RF = \frac{R_0}{R_t} \quad (1)$$

2.4. Permeation experiments on the pre-screened enhancers

2.4.1. Preparation of solution

Phosphate buffer (PB, pH–7.4, monosodium phosphate monohydrate and disodium phosphate heptahydrate concentrations of