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COMMUNICATION

Effect of Dimethylacetamide and 2-Pyrrolidone on the Iontophoretic Permeability of LHRH Through Porcine Skin

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ABSTRACT

The effect of penetration enhancer (PE) and iontophoresis on the in vitro permeability of luteinizing hormone releasing hormone (LHRH) through porcine epidermis was investigated. The permeability coefficient of LHRH increased ($p < 0.05$) through PE (e.g., dimethylacetamide [DMAC] and 2-pyrrolidone [2-P])-treated epidermis. Iontophoresis further increased the permeability of LHRH ($p < 0.05$) through the PE-pretreated epidermis in comparison to the control (without PE-treated epidermis). This shows that iontophoresis can synergize with PEs such as DMAC and 2-P to provide an additional driving force to maintain and control the target flux of LHRH.

INTRODUCTION

Peptides and proteins, because of their hydrophilic nature and large molecular size, have limited permeability in the skin. The utility of iontophoresis to overcome some of the obstacles of peptide delivery have been widely reported (1). Iontophoresis in combination with chemical penetration enhancer (PE) can be employed to increase the permeability of peptides through the skin (2). The combining effect of iontophoresis and PE may permit the use of lower quantities of enhancer and current within the delivery system and potentially circumvent adverse reactions, irreversible structural changes into the skin, and dermatotoxicity. A synergism of iontophoresis and ethanol as PE was reported on the transport of peptides through human epidermis (3). It has been shown that a 2-hr pretreatment with absolute ethanol followed by iontophoresis dramatically increased the permeability coefficient of a high molecular weight polypeptide such as insulin through human skin (4).

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Recently, oleic acid in combination with ethanol followed by iontophoresis was shown to increase the permeability of LHRH (5).

LHRH has a short elimination half-life of 4.55 min (6) and therefore it is a potential drug to be administered transdermally at controlled rate. Dimethylacetamide (DMAC) and 2-pyrrolidone (2-P) have been shown to enhance the penetration of several components (7,8). In this study, we have investigated the effect of PE (DMAC and 2-P) pretreatment and iontophoresis on the in vitro permeability of [^{3}H] LHRH through porcine epidermis. The PE pretreatment of the porcine epidermis step represents the effect of PE (3).

MATERIALS AND METHODS

Chemicals

[^{3}H] Luteinizing hormone releasing hormone (LHRH, specific activity 51 Ci/mmol) was obtained from NEN Research Products, DE. Dimethylactamide, 2-pyrrolidone, *n*-butanol, acetic acid, and NaCl (Sigma Chemical Co., St. Louis, MO) were used. All other chemicals used were of analytical grade. Deionized water (resistivity $\geq$ 18 Ω cm) was used to prepare all solutions. The purity of radiolabeled LHRH was evaluated, prior to use, by thin-layer chromatography (TLC). The radiochemical purity of the peptide was 99%. We also checked the peptide degradation by TLC during the course of iontophoresis experiments. The peptide was almost intact (>92%), therefore electrochemical degradation of the peptide was minimal.

Preparation of Epidermis

Porcine ears were obtained from local slaughter house and after they were cleaned under cold running water, the outer region of the ear was cut. The whole skin was removed carefully from the underlying cartilage with the help of a scalpel. The epidermis was prepared by soaking the whole skin in water at 60°C for 45 sec. The skin was removed from water, blotted dry, and pinned with dorsal side down. The intact epidermis was teased off from dermis with forceps, washed with water, and used in the in vitro permeability studies (9). For pretreatment, the epidermis was immersed in the PE for 2 hr. Epidermis without pretreatment was used as control.

In-Vitro Studies

Franz diffusion cells modified for iontophoresis were used in all transport studies. The control/pretreated epidermis was sandwiched between the cells with stratum corneum facing the donor compartment. The maximum capacity of the donor and receiver compartment was 3.5 ml and 7 ml, respectively. The surface of epidermis exposed to the solution was 3.14 cm^2. The donor compartment contained 2 ml of LHRH solution (0.2 μCi/ml of LHRH in normal saline [0.9% NaCl solution]) and the receiver compartment contained 7 ml of normal saline. The cells were maintained at 37 $\pm$ 0.5°C by PMC Dataplate® stirring digital dry block heater (Crown Bioscientific Inc, NJ). The content of the receiver compartment was stirred with a magnetic bar at 100 rpm. At specified intervals, 1-ml samples were withdrawn from the receiver compartment and an equivalent amount of normal saline (1 ml) was added to maintain the constant volume. Ag/AgCl electrodes (99.99+% Ag wire plated with AgCl) of 0.5 mm diameter and 4 cm length (Keltronics Corp., OK) were used in the study. These electrodes are non-polarizable, reversible, and therefore do not decompose water. Anode was placed in the donor and cathode in the receiver for anodal iontophoresis. LHRH in normal saline was positively charged. The constant current of 0.2 mA/cm^2 was applied with the Scepter® (Keltronics Corp.) which is a flexible programmable voltage/current source that permits facile checking of voltage/current throughout the experiment. The results were expressed as the mean $\pm$ SD of three experiments. The samples were assayed by liquid scintillation counting. Each sample was mixed with 10 ml of scintillation cocktail (econosafe®, biodegradable counting cocktail, Research Products International Corp., IL) and counted in a liquid scintillation counter (Packard Instruments, Tri Carb® 2100 TR, CT). The instrument was programmed to give counts for 10 min.

Data Analysis

The cumulative amount of LHRH permeated per unit skin surface area was plotted against time, and slope of the linear portion of the plot was estimated as steady state flux (J_{ss}). The permeability coefficient (K_p) was calculated as (10)

$$K_p = J_{ss}/C_v$$

where C_v is the total donor concentration of the solute. Statistical comparisons were made using Student's *t*-test. The level of significance was taken as $p < 0.05$.

RESULTS AND DISCUSSION

The effect of PE and iontophoresis on the in vitro permeation profiles of LHRH through the epidermis is

shown in Fig. 1. The iontophoretic transport was greater than passive through the PE-treated and control epidermis. The permeability coefficient and enhancement factors are shown in Fig. 2 and Table 1, respectively. The permeability coefficient of LHRH during iontophoresis was significantly greater ($p < 0.05$) than passive diffusion through control epidermis. Enhancement in the permeability coefficient of LHRH was 1.31 and 2.31 times greater through DMAC- and 2-P-pretreated epidermis, respectively, than the control.

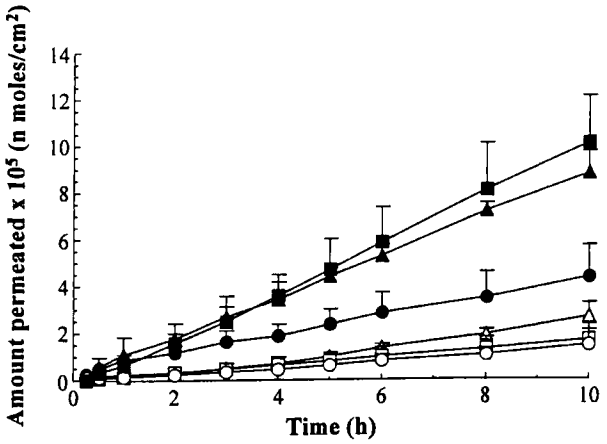

Figure 1. The effect of PE and iontophoresis on the in vitro transport of LHRH through porcine epidermis. Each data point is the mean ± SD of three determinations: Key: (○) control (passive); (●) control (iontophoresis); (□) 25% dimethylacetamide (passive); (■) 25% dimethylacetamide(iontophoresis); (△) 2-pyrrolidone (passive); (▲) 2-pyrrolidone (iontophoresis).

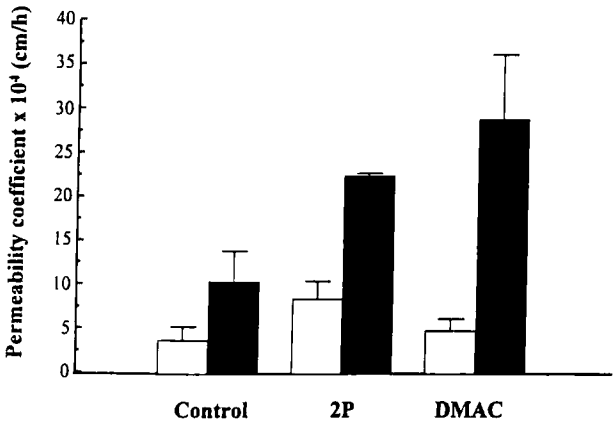

Figure 2. Permeability coefficient of LHRH through porcine epidermis after PE pretreatment and iontophoresis. Key: (□) passive; (■) iontophoresis; control = normal saline; DMAC = dimethylacetamide; 2-P = 2-pyrrolidone.

Table 1

Enhancement Factors of LHRH Due to Penetration Enhancer and Iontophoresis.

Penetration Enhancer (PE)	Enhancement Factor		
	E_1	E_2	E_3
Control	—	—	—
DMAC	1.31	2.80	8.04
2-P	2.31	2.18	6.27

DMAC = dimethylacetamide, 2-P = 2-pyrrolidone.

$$E_1 = \frac{\text{Permeability coefficient with PE (passive)}}{\text{Permeability coefficient without PE (passive)}}.$$

$$E_2 = \frac{\text{Iontophoretic permeability coefficient with PE}}{\text{Iontophoretic permeability coefficient without PE}}.$$

$$E_3 = \frac{\text{Iontophoretic permeability coefficient with PE}}{\text{Permeability coefficient without PE (passive)}}.$$

Iontophoresis increased the transport of LHRH through the PE-pretreated epidermis. DMAC, and 2-P pretreatments of the epidermis significantly enhanced ($p < 0.05$) the iontophoretic permeability coefficient of LHRH compared to control. Also, significant enhancement ($p < 0.01$) in the iontophoretic permeability of LHRH was observed through DMAC- and 2-P-pretreated epidermis in comparison to the passive permeability through the control. Enhancement of 2.80 and 2.18 in the iontophoretic permeability of LHRH through DMAC- and 2-P-pretreated epidermis, respectively, was obtained in comparison to iontophoresis through the control epidermis. This shows that iontophoresis can synergize with PEs such as DMAC, and 2-P to provide an additional driving force to maintain and control the target flux. The PE would moderate the iontophoretic regimen required to achieve the target flux, thus improving the tolerability of the skin to the iontophoretic regimen. Electrical current could also be used to modulate the transport over and above the effect of a PE. Modulation of the input is necessary in the administration of some polypeptides to produce the desired pharmacological action (11).

In summary, our work identifies the synergism between PEs (e.g., DMAC and 2-P) and iontophoresis as a technique to enhance and control the transdermal delivery of LHRH. An appropriate combination of PE and iontophoresis can produce the targeted increase in the flux of LHRH in a synergistic manner.

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