Drug Development and Industrial Pharmacy, 23(11), 1111-1114 (1997)

COMMUNICATION

Percutaneous Absorption of LHRH Through Porcine Skin: Effect of N-Methyl 2-Pyrrolidone and Isopropyl Myristate

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ABSTRACT

The effect of penetration enhancer (PE) on the in vitro permeability of luteinizing hormone-releasing hormone (LHRH) through porcine epidermis was investigated. The permeability coefficient of LHRH significantly increased ($p < 0.05$) through PE-(e.g., n-methyl 2-pyrrolidone [NMP], and isopropyl myristate [IPM]) treated epidermis in comparison to the control (without PE-pretreated epidermis). The higher permeability coefficient was observed with NMP followed by IPM. This shows that PE such as NMP and IPM can enhance the percutaneous absorption of peptides such as LHRH.

INTRODUCTION

In the last decade, special attention has been paid to understanding the mechanisms involved in the percutaneous penetration of the peptide and protein drug. Transdermal administration of drug promises several advantages over oral or parenteral administration of the peptides. Absorption via the transdermal route is limited by the generally poor penetration of the drugs through the stratum corneum (SC), the outermost layer of the skin that comprises keratin-rich dead cells embedded in a complex lipid matrix. This is especially true for peptide and protein drugs which, because of their hydrophilic nature and large molecular size have limited permeability in the skin. Reducing the barrier properties of the SC by using penetration enhancers (PE) is one method by which drug absorption can be improved (1).

N-Methyl 2-pyrrolidone (NMP) has been used to accelerate the permeation of a nonsteroidal anti-inflammatory drug, mefanamic acid, across rabbit skin in vivo (2). NMP in combination with propylene glycol enhanced the transport of naloxone through human skin (3). Isopropyl myristate (IPM) increased the permeation of naproxen through shed snake skin (4). Also, by com-

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bining NMP with lauryl-2pyrrolidone in isopropyl myristate as a vehicle for transdermal delivery, the permeation of phenol red and 5-flouorouracil was significantly increased (5). Furthermore, NMP in combination with IPM increased metronidazole penetration through human skin (6). However, the effect of NMP and IPM as PE on the percutaneous absorption of peptide and protein drugs has not been well investigated.

Luteinizing hormone-releasing hormone (LHRH) is used clinically for inducing ovulation in women with hypothalamic amenorrhea, inducing puberty and spermatogenesis in men with hypogonadotrophic hypogonadism, and the treatment of prostatic carcinoma. It has also been used in endometriosis and polycystic ovary syndrome (7). In this study, we have investigated the effect of PE (NMP and IPM) pretreatment 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 (8).

MATERIALS AND METHODS

Chemicals

[^{3}H] LHRH (specific activity 51 Ci/mmol) was obtained from NEN Research Products, DE. NMP, IPM, 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 MΩ) 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%.

Preparation of Epidermis

Porcine ears were obtained from local slaughter house and after the ears were cleaned under cold running water, the outer region was cut. The whole skin was removed carefully from the underlying cartilage with 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 were used in all transport studies. The control/pretreated epidermis was sandwiched between the cells with SC facing the donor compartment. The maximum capacity of each 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 contents 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. The dilution of receiver compartment was taken into account when evaluating the permeation data. 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, 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 one way ANOVA. The level of significance was taken as $p < 0.05$.

RESULTS AND DISCUSSION

The effect of PE on the in vitro permeation profiles of LHRH through the control and PE-pretreated epidermis is shown in Fig. 1. NMP and IPM led to an in-

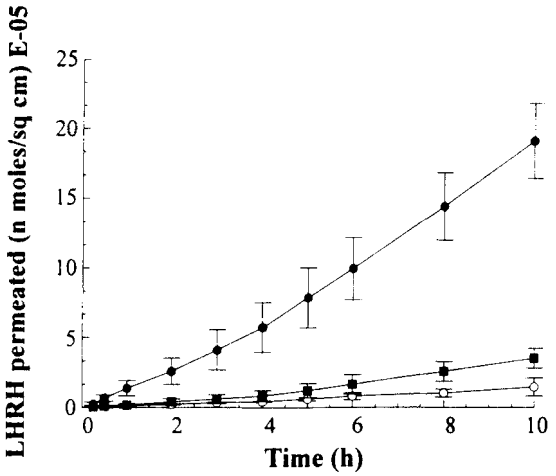

Figure 1. The effect of PE on the in vitro transport of LHRH through porcine epidermis. Each data point is the mean ± SD of three determinations.
Key: (○) control (normal saline)
(●) N-methyl 2-pyrrolidone
(■) isopropyl myristate.

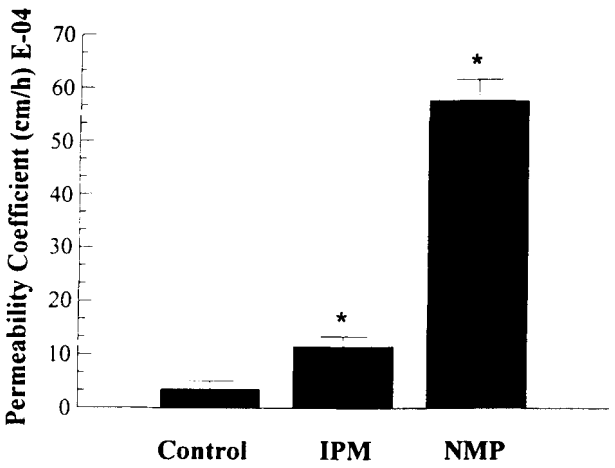

Figure 2. Permeability coefficient of LHRH through porcine epidermis after PE pretreatment. Control = normal saline; NMP = N-methyl 2-pyrrolidone; IPM = isopropyl myristate; * Statistically significant ($p < 0.05$).

crease in the LHRH transport as compared to the control. While evaluating the effectiveness of a permeation enhancer the skin is often pretreated with the enhancer before applying the drug. This method of application enables direct assessment of enhancer activity without the results being affected by the formulation. The permeability coefficient and enhancement factors are shown in Fig. 2 and Table 1, respectively. NMP and IPM pretreatments of the epidermis significantly ($p < 0.05$) increased the permeability coefficient of LHRH as compared to the control. Enhancement in the permeability coefficient of LHRH was 16.18 and 3.21 times greater through NMP and IPM pretreated epidermis, respectively, than the control.

It is often stated that there is no universal enhancer for all drugs. Thus each transdermal drug delivery system must be designed around the drug it contains to optimize the therapeutic effect of the active ingredient. It has been proposed that NMP displaced bound stratum corneum water and thus increased the penetration of lidocaine. It was also observed that N-alkyl pyrrolidones appeared to exhibit a relationship wherein unsaturated groups were more effective than saturated ones in promoting naloxone flux (11). Furthermore, NMP increased with time the chemical potential and thermodynamic activity of the drug flurbiprofen and increased its flux by changing the diffusional resistance of the membrane (12). The primary site of action of NMP is considered to be polar route, and the hydration of the skin owing to their intrinsic humectant activity, is believed to be the significant factor in its effectiveness.

IPM on the other hand, acts by disrupting the lipid barrier of the skin. IPM possesses both polar and nonpolar characteristics. It was observed that IPM treatment of the skin increased the transport of ionized naproxen significantly more than that of unionized, indicating a significant reduction of the barrier function of the stratum corneum by IPM (4). Modification of polar pathways in the stratum corneum by interaction with the

Table 1

Enhancement Factors of LHRH due to Penetration Enhancer

Penetration Enhancer (PE)	Enhancement Factor
Control	–
NMP	16.18
IMP	3.21

NMP = *n*-methyl 2-pyrrolidone.
IPM = isopropyl myristate.

$$\text{Enhancement factor} = \frac{\text{Permeability coefficient with PE}}{\text{Permeability coefficient without PE}}$$

intracellular proteins could be one of the mechanisms involved by which IPM causes the enhancement in the transport of the drugs.

In summary, these investigations show that PE such as NMP and IPM can be used to enhance the transdermal delivery of large molecular weight peptide such as LHRH.

ACKNOWLEDGMENT

We acknowledge the financial support from NSF through ND/EPSCoR # 4817.

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