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COMMUNICATION

Enhancing Effects of Fatty Acids on Piroxicam Permeation Through Rat Skins

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ABSTRACT

To increase the skin permeation of piroxicam from the Poloxamer 407 gel, fatty acid was added as a penetration enhancer to the Poloxamer 407 gel containing 1% piroxicam. The enhancing effects of the enhancer on the skin permeation of piroxicam were evaluated using Franz diffusion cells fitted with intact excised rat skins. To elucidate the modes of the action of enhancers, thermal analysis and histological examinations were conducted. Among fatty acids tested, linoleic acid showed the highest enhancing effects, with an enhancement factor (EF) of 1.76. From the thermal analysis results, fatty acids have fluidizing effects on the stratum corneum. The skin pretreated with the Poloxamer 407 gels containing piroxicam including linoleic acid showed a loosely layered stratum corneum and wide intercellular space.
Key Words: *Enhancing effects; Fatty acids; Permeation; Piroxicam; Poloxamer gels.*

INTRODUCTION

The skin is widely recognized for its outstanding barrier properties compared with other biological membranes. The low permeability of the skin relative to other biological tissues is well known and keeps the skin as a minor port of entry for drugs. To improve the permeability of drugs through the skin, penetration enhancers have been incorporated into a formulation that would reversibly reduce the barrier resistance of the skin and thus allow the drug to penetrate to the viable tissues and enter the systemic circulation. Significant improvements can be made by optimizing the thermodynamic activity of the drug in the formulation and with the judicial use of permeation enhancers (1).

The therapeutic efficacy of a topically applied drug depends on its ability to penetrate the skin and be accumulated in the deeper layers of the skin. The extent of this

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absorption varies depending on both the physiochemical properties of the penetrant and its formulation. The vehicle composition can affect both drug release and skin permeability properties (2).

In this laboratory, the physicochemical characteristics of piroxicam in Poloxamer gel (3) and thermorheological behavior of Poloxamer 407 solution (4) were studied. To increase the skin permeation of piroxicam from the Poloxamer 407 gel, fatty acids as penetration enhancers were added to the Poloxamer 407 gel containing 1% piroxicam. The objective of this investigation was to determine the feasibility of topical delivery of piroxicam containing fatty acid as an enhancer by studying its in vitro permeation characteristics across rat skin. The enhancing effects of the enhancers on the skin permeation of piroxicam were evaluated using Franz diffusion cells fitted with intact excised rat skins. To elucidate the modes of action of fatty acid, thermal analysis and histological examinations were conducted.

EXPERIMENTAL

Materials

Piroxicam was received from Chodang Pharmaceutical Company (Seoul, Korea). Poloxamer 407 and 188 were received from BASF (Ludwigshafen, Germany). Hydroxypropyl methylcellulose was a gift of Handok Pharmaceutical Company (Seoul, Korea). Linoleic acid, lauric acid, oleic acid, and capric acid were purchased from Sigma Chemical Company (St. Louis, MO). All other reagents were analytical grade and were used without further purification.

Preparation of Piroxicam-Poloxamer Gels Containing Enhancer

For preparation of the gels, 20 g of Poloxamer were added to water with gentle stirring, and the solution was left overnight in a refrigerator to complete polymer desolvation. To stabilize the gel system, 0.5 g of hydroxypropyl methylcellulose was added as an emulsifying agent. To the cold Poloxamer solution, 1 g of piroxicam and 5 g of fatty acid dissolved in 40 ml of propylene glycol were added with stirring. The preparation was then brought to 100 ml with the water and stored in a shaking water bath at 30°C for 2 days.

Skin Permeation Study

The freshly excised full-thickness skin was mounted on the diffusion cell with the stratum corneum side facing the donor compartment and the dermal side facing the receptor compartment. Poloxamer gel (2 g) was placed in intimate contact with the skin, and the donor cap was covered with a parafilm and clamped to study the effect of enhancer on the drug release. The sampling port was sealed with a parafilm to prevent the evaporation of the receptor medium. The Sorensen's phosphate buffer (pH 7.4) was then introduced into the receptor compartment, which was maintained at 37°C by a circulating water bath. The donor compartment was maintained at the ambient temperature of 25°C ± 1°C. The samples from the receptor were taken at predetermined time intervals and immediately replaced by an equal volume of fresh buffer solution. The sample taken from the receptor was analyzed at 320 nm by an ultraviolet (UV) spectrophotometer.

Thermal Analysis of the Stratum Corneum Incubated with Enhancer

Rat skins were pretreated with 5% enhancer in propylene glycol for 12 hr. Then, rat skins were wiped off with tissue and soaked in trypsin solution with stirring to eliminate fat tissues. The stratum corneum layer was separated with forceps very carefully and was dried with reduced pressure. Dried stratum corneum was sealed into alumina pans and analyzed with thermal gravimetric/dynamic thermal analyses (TG/DTA) (Seiko SSC 5200, Tokyo, Japan). The skin untreated with enhancer served as a control.

Histological Examination of Excised Stratum Corneum

Histological changes in the stratum corneum were examined by applying the Poloxamer gels containing an enhancer to rat abdominal skins. Poloxamer gels containing enhancer were applied on the excised rat skins mounted on the diffusion cell for 24 hr. Then, gels applied on the rat skin were wiped off with tissue, and the skins were fixed in 10% formalin by the conventional procedure, stained with hematoxylin-eosin, and examined under a microscope (5). The skin untreated with an enhancer served as a control.

RESULTS AND DISCUSSION

Permeation Profiles of Piroxicam from the Poloxamer 407 Gels Containing Enhancers

To increase the skin permeation of piroxicam, various fatty acids were incorporated into Poloxamer gels. Linoleic acid, capric acid, and oleic acid as unsaturated fatty

acids and lauric acid as saturated fatty acid were used as enhancers. The cumulative amount of piroxicam permeating the skin was plotted against time (Fig. 1). A linear profile (steady state) was observed during the 24 hr, and the slope of the linear portion of the curve was determined by linear regression. Permeation rate (μg/cm^2 per hr) at steady state was calculated by dividing the slope of the linear portion of the curve by the area of the skin surface through which permeation took place. The decreasing order of the enhancement effect was seen as follows: linoleic acid, oleic acid, capric acid, and lauric acid. Poloxamer gel containing piroxicam including fatty acid as an enhancer is a good preparation to promote the percutaneous absorption of drugs. It has been reported that the various enhancers, such as saturated long-chain fatty acids, incorporated into carbopol gels for capsaicin, sodium novivamide, and lauric acid showed the increased permeation of sodium novivamide (6).

The effectiveness of penetration enhancers was determined by comparing the permeation rate of piroxicam in the presence and absence of enhancers (Table 1). This was defined as the enhancement factor (EF). Among fatty acids tested, linoleic acid showed the highest enhancing effects, with enhancement factor of 1.76.

Table 1

Enhancement Factor of the Various Fatty Acids

Enhancer	Permeation Rate (μg/cm^2 per hr)	EF
Control	5.11 ± 0.52	1
Lauric acid	6.38 ± 0.62	1.25
Linoleic acid	8.99 ± 0.78	1.76
Capric acid	6.79 ± 0.65	1.33
Oleic acid	8.43 ± 0.75	1.65

The value represents the mean ± SD (n = 3).

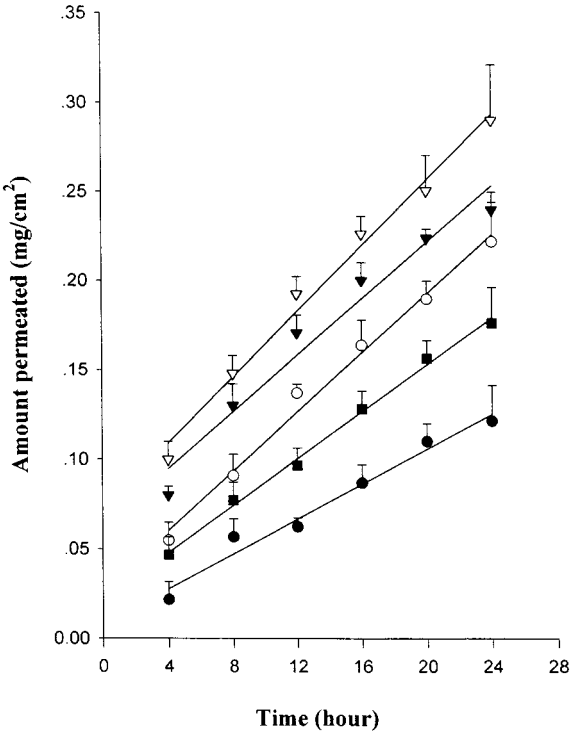


Figure 1. Effect of fatty acids in the Poloxamer gel containing 1% piroxicam on the drug permeation through excised rat skins: ●, control; ■, lauric acid; ▼, oleic acid; ○, capric acid; ▽, linoleic acid.

Thermal Analysis for Study of Lipid Fluidity in Stratum Corneum

The skin barrier function is known to reside in the stratum corneum. One of the techniques to study the physicochemical properties of the stratum corneum barrier is thermal analysis (7). To gain information on the mode of action of the agents, the ability of the penetration enhancers to affect the degree of order of the lipid bilayers in the horny layer has been assessed by DTA and correlated with the penetration-enhancing effect (8). It has been proposed that the differential scanning calorimetry (DSC) peaks near 65°C, 75°C, and 105°C for human and porcine stratum corneum were due to the thermal transitions involving intercellular lipids, lipid-protein complexes, and intercellular keratin, respectively (9,10). The thermogram of stratum corneum untreated with en-

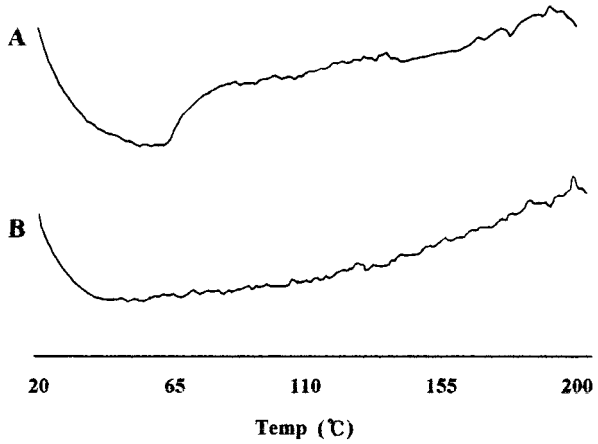
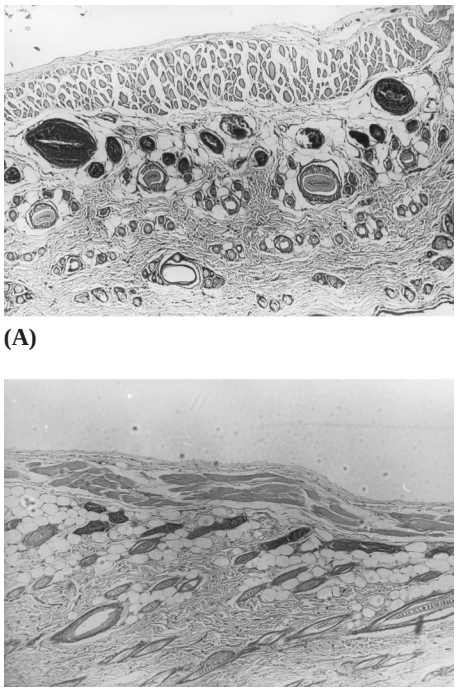


Figure 2. DTA curves of the rat stratum corneum pretreated with linoleic acid: (A) control; (B) linoleic acid.



(A)

(B)

Figure 3. Histological micrographs of the rat skin pretreated with the piroxicam-Poloxamer gel containing linoleic acid: (A) control; (B) linoleic acid.

hancer showed a small, broad endothermic peak near 57.5°C having the DTA value of 10.37 μV.

Among fatty acids tested, linoleic acid showed the highest enhancing effects, with an EF of 1.76 (Table 1 and Fig. 1). The thermograms of stratum corneum treated with linoleic acid were studied. The stratum corneum treated with linoleic acid showed the broad endothermic peak of the DTA value −0.55 μV near 44.6°C. When compared to intact stratum corneum, the sharpness of the endothermic peak was decreased. The results of thermal analysis for rat skins indicated that various enhancers had different fluidizing effects on lipids of the stratum corneum (Fig. 2). The changes in the thermal profile seen with fatty acid–treated samples suggest that its incorporation into the stratum corneum resulted in decreased lipid order. The results of this study showed that fatty acid has fluidizing effects on stratum corneum.

Histological Examination of the Stratum Corneum

To study the effects of an enhancer in piroxicam penetration through rat skin, Poloxamer gels containing linoleic acid were applied on excised rat skin mounted on a diffusion cell for 12 hr. As a control, another rat skin was also pretreated with normal saline for 12 hr. Intact skin is composed of stratum corneum, epidermis, dermis, and subcutaneous fats and has well-weavered structures. The skin pretreated with the Poloxamer 407 gels containing linoleic acid showed that stratum corneum was loosely layered, and intercellular spaces were wide (Fig. 3).

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