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Effects of non-ionic surfactants as permeation enhancers towards piroxicam from the poloxamer gel through rat skins

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Abstract

The enhancing effects of non-ionic surfactants on the permeation of piroxicam from the poloxamer gels were evaluated using Franz diffusion cells fitted with excised rat skins. The effectiveness of penetration enhancers, the ratio of piroxicam flux in the presence or absence of enhancers, was defined as the enhancement factor. Among the various non-ionic surfactants tested, polyoxyethylene-2-oleyl ether showed the highest enhancing effects with an enhancement factor of 2.84. To elucidate the mechanisms of the action of enhancers, thermal analysis and histological examinations were carried out. Thermal analysis reveals that various surfactants have different fluidizing effects on stratum corneum. Skin pretreated with the poloxamer 407 gels containing various surfactants showed a loosely layered stratum corneum and wide intercellular space.

Keywords: Permeation; Surfactants; Piroxicam; Poloxamer; Permeation enhancer; Rat skin

1. Introduction

The transdermal route has many advantages for administration of drugs in local and systemic therapy. But, 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 makes the skin a minor port of entry for drugs. The use of penetration enhancers is a promising way to enhance the permeation of drugs (Franz et al., 1992; Hadgraft et al., 1973). Ideally, these enhancers reversibly reduce the barrier effects of the stratum corneum and allow the drug to penetrate the viable tissues, facilitating systemic circulation (Southwell and Barry, 1983). In previous studies from this laboratory, the physicochemical characteristics of piroxicam in poloxamer gel (Shin and Cho, 1997) and thermorheological behavior of poloxamer 407 solution (Cho et al., 1997) were studied. To increase the skin permeation of piroxicam (pK_a 5.1) (Drug Information, 1988) from the poloxamer 407 gel, non-ionic surfactants as penetration enhancers were added to the piroxicam–poloxamer 407 gel. The objective of this study was to determine the

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feasibility of topical delivery of piroxicam-containing surfactants 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 studied using Franz diffusion cells fitted with intact excised rat skins. In order to elucidate the modes of the action of surfactants, thermal analysis and histological examinations were conducted.

2. Materials and methods

2.1. Materials

Piroxicam was received from Chodang Pharm. Co. (Seoul, South Korea) and hydroxypropyl methylcellulose (HPMC) was a gift from Handok Pharm. Co. (Seoul, South Korea). Poloxamer 407 was received from BASF (Ludwigshafen, Germany). Polyoxyethylene-23-lauryl ether, polyoxyethylene-2-oleyl ether and polyoxyethylene-2-stearyl ether, as enhancers, were purchased from Sigma Chemical Co. (St. Louis, MO). Monobasic sodium phosphate and dibasic sodium phosphate were of analytical grade. All other reagents were analytical grade and were used without further purification.

2.2. Methods

2.2.1. Preparation of piroxicam–poloxamer gels containing various enhancers

The cold technique was used for preparation of gels. Poloxamer (20 g) was added into cold water with gentle stirring, and the solution was left overnight in a refrigerator to complete polymer desolvation. HPMC (0.5 g) was added as an emulsifying agent to stabilize the gel system. Piroxicam (1 g) and the surfactants (5 g) dissolved in 40 ml of propylene glycol were added, while stirring, to the cold poloxamer solution and then left overnight in a refrigerator to complete mixing of the solutions. The preparation was then made up to 100 ml with water and stored in a shaking water bath at 30°C for 2 days.

2.2.2. 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 gels (2 g) were placed in intimate contact with the skin and the donor cap was covered with parafilm and clamped to study the effect of an enhancer on drug release. The sampling port was sealed with parafilm to prevent the evaporation of the receptor medium. The receptor solution (pH 7.4 Soerensen's phosphate buffer) was then introduced into the stirred receptor compartment maintained at 37°C by a circulating water bath. The donor compartment was maintained at the ambient temperature of 25 ± 1°C. The samples from the receptor compartment were withdrawn at predetermined time intervals and immediately replaced by an equal volume of fresh buffer solution. Initial experiments confirmed the maintenance of sink condition by this procedure. The samples withdrawn from receptor compartment were then analyzed at 320 nm.

2.2.3. Thermal analysis

The rat skins were pretreated with 5% non-ionic surfactants in propylene glycol for 12 h. Then, rat skins were wiped off with tissue, soaked in trypsin solution with stirring to eliminate fat tissues. The stratum corneum layer was separated with forceps very carefully and was dried under reduced pressure. The samples of the stratum corneum incubated in 5% of various enhancers in propylene glycol were sealed into alumina sample pans and analyzed with TG/DTA instruments (Seiko SSC, Japan). The temperature was calibrated using indium standards. The operating conditions in an open-pan system were as follows: sample weight, 5–10 mg; heating rate, 10°C/min under purging N_2 at 70 ml/min. The temperature was increased from 20 to 200°C and then allowed to decrease to 20°C. Skin not treated with any enhancer served as a control.

2.2.4. Histological examination

Poloxamer gels containing an enhancer and piroxicam were applied for 24 h on the excised rat skins mounted on the diffusion cell. Then, gels

applied on the rat skin were wiped off with tissue, fixed in 10% formalin by the conventional procedure, stained with hematoxylin–eosin, and examined under a microscope (Aioi et al., 1993). Skin not treated with any enhancer served as a control.

3. Results and discussion

3.1. Permeation of piroxicam from the poloxamer 407 gels containing enhancers

The effects of non-ionic surfactant as permeation enhancer on the permeation of piroxicam from poloxamer gel formulation across the rat skin were investigated.

Fig. 1 shows a permeation profile of piroxicam from the poloxamer 407 gel including non-ionic surfactants such as polyoxyethylene-23-lauryl ether, polyoxyethylene-2-oleyl ether and polyoxyethylene-2-stearyl ether as enhancers. The cumulative amount of piroxicam penetrating the skin was plotted against time. A linear profile (steady state) was observed during the 24 h period and the slope of the linear portion of the curve was determined by linear regression. The permeation rate ($\mu g/cm^2/h$) 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.

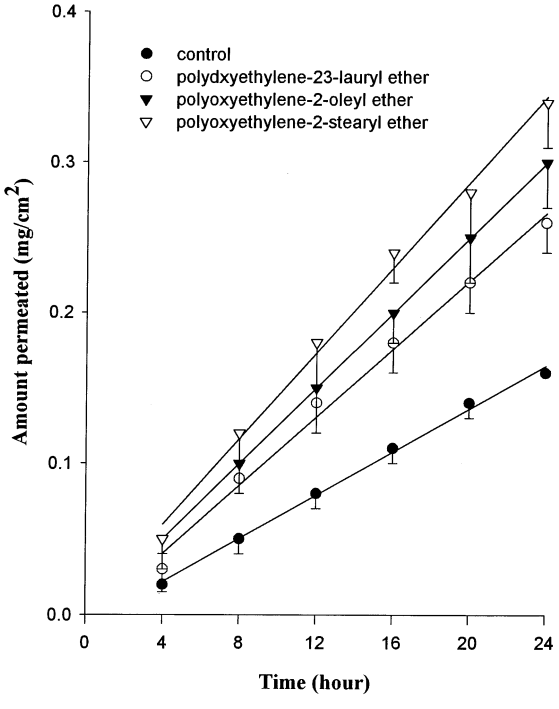

Fig. 1. Effect of non-ionic surfactants in the poloxamer gel containing 1% piroxicam on drug permeation through excised rat skins.

Table 1
Enhancement factor of the various non-ionic surfactants

Enhancer	Permeation rate ($\mu g/cm^2/h$)	EF
Control	5.11 ± 0.52	1
Polyoxyethylene-23-lauryl ether	11.12 ± 0.98	2.18
Polyoxyethylene-2-oleyl ether	14.49 ± 1.28	2.84
Polyoxyethylene-2-stearyl ether	13.39 ± 1.25	2.62

Each value represents the mean $\pm$ SD of three determinations.

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).

$$EF = \frac{\text{permeation rate of piroxicam at steady state in the presence of enhancers}}{\text{permeation rate of piroxicam at steady state in the absence of enhancers}}$$

The permeation of piroxicam from the poloxamer 407 gel containing piroxicam including the enhancers showed enhanced permeation and shortened lag time. The order of the enhancement effect was polyoxyethylene-2-oleyl ether, polyoxyethylene-2-stearyl ether, polyoxyethylene-23-lauryl ether. Among the non-ionic surfactants tested, polyoxyethylene-2-oleyl ether showed the highest enhancing effects, with an EF of 2.84 (Table 1; Fig. 1).

Poloxamer gels containing piroxicam including surfactants as enhancers are good preparations to promote the percutaneous absorption of drugs.

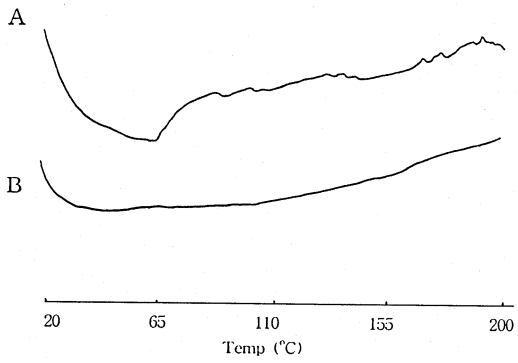

Fig. 2. DTA curves of the rat stratum corneum pretreated with enhancer: (A) control; (B) polyoxyethylene-2-oleyl ether.

3.2. Thermal analysis

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 (Tanojo et al., 1994). To gain information on the mode of action of the agents, the ability of the putative penetration enhancers to affect the degree of order of the lipid bilayers in the horney layer has been assessed by DTA and correlated with the penetration enhancing effect (Carelli et al., 1993).

To elucidate the mechanism of the action of various enhancers, thermal analysis and histological examinations were conducted. It has been proposed that the DSC peaks near 65, 75 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 (Golden et al., 1986, 1987). The thermogram of stratum corneum not treated with the enhancer showed a small broad endotherm near 57.5°C having the DTA value of 10.37 μV. When compared to this intact stratum corneum, the thermogram of the stratum corneum incubated in propylene glycol showed an endothermic peak with the DTA value -9.80 μV only near 59.5°C and shifted to a slightly higher temperature than intact stratum corneum. When compared to intact stratum corneum, the sharpness of the endothermic peak decreased. But no significant differences were found. The stratum corneum incubated with 5% polyoxyethylene-2-oleyl ether in propylene glycol had a broad endothermic peak with the DTA value of 45.4°C. There was a thermal transition starting from 30.5°C. The DTA curve of the stratum corneum incubated with polyoxyethylene-2-oleyl ether in propylene glycol was very smooth.

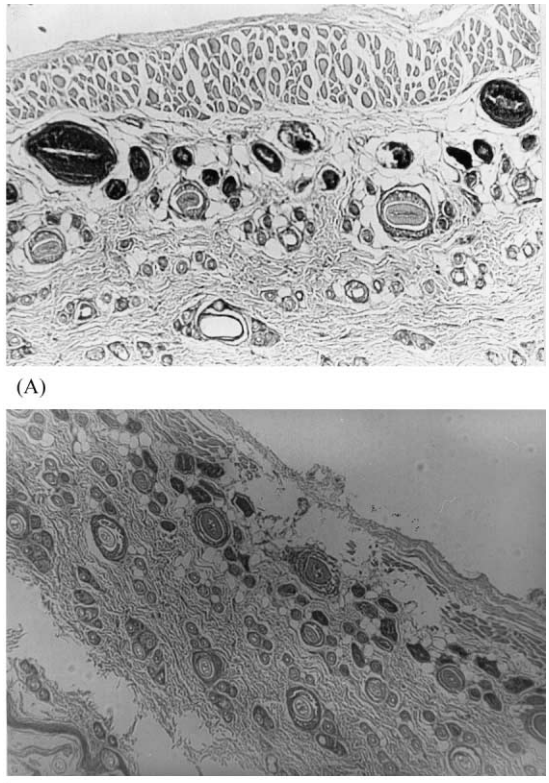

Fig. 3. Histological micrographs of the rat skin pretreated with the piroxicam–poloxamer gel containing: (A) control; (B) polyoxyethylene-2-oleyl ether.

This coincides with the fact that the permeated amount of piroxicam from the poloxamer 407 gel containing polyoxyethylene-2-oleyl ether is very high (Fig. 1). The changes in the thermal profile seen with surfactants-treated sample suggest that its incorporation into the stratum corneum resulted in decreased lipid order. The results of thermal analysis indicated that various enhancers had different fluidizing effect on the lipids of the stratum corneum (Fig. 2).

3.3. Histological examination

To study the role of enhancers in piroxicam penetration through rat skin, poloxamer gels containing polyoxyethylene-2-oleyl ether were applied on the excised rat skin mounted on the diffusion cell for 12 h. As a control, another rat skin was also pretreated with normal saline for 12 h.

Intact skin is composed of stratum corneum, epidermis, dermis and subcutaneous fats and has well-woven structures. The skin pretreated with the poloxamer 407 gels containing the enhancer and piroxicam showed that the stratum corneum was loosely layered and that intercellular spaces were wide (Fig. 3).

Acknowledgements

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