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Enhanced Transdermal Delivery of Furosemide from the EVA Matrix through the Rat Skin

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ABSTRACT – This study was performed to examine the possibility of increasing the level of furosemide permeation from the ethylene-vinyl acetate (EVA) matrix through the skin by incorporating various enhancers in the EVA matrix. The effects of the enhancers on the level of furosemide permeation through the skin were evaluated using Franz diffusion cells with intact excised rat skins. The enhancers examined were the fatty acids (saturated, unsaturated), the pyrrolidones, the propylene glycol derivatives, the glycerides and the non-ionic surfactants. Among the enhancers used, polyoxyethylene-2-oleyl ether (a non-ionic surfactant) showed the best enhancement. The polyoxyethylene 2-oleyl ether as a permeation enhancer could be used for development of furosemide-EVA transdermal matrix system.

Key words – Furosemide, Ethylene-vinyl acetate, Transdermal delivery, Penetration enhancer, Matrix, Skin permeation

Furosemide, one of potent diuretics, has been used for the treatment of edema. In the case of oral application, it can induce the side effects like polyurea, dizziness, dry mouth, nausea, gastric disturbances and so on. To avoid the systemic side effects and gastric disorders that could be occurred after oral administration, alternative routes of administration have been considered. Percutaneous delivery of NSAIDs has advantages of avoiding hepatic first pass effect and delivering the drug for extended period of time at a sustained level.

The skin is an attractive potential route for drug administration because it can avoid the first-pass hepatic metabolism of a drug. This route can potentially allow decreased drug doses with reduced side effects.[1,2] The transdermal route for systemic drug delivery has attracted considerable attention.[3-6] However, a major problem encountered using this administration route is the low permeability through the skin. Several penetration enhancers,[1] prodrugs,[7] iontophoresis,[8] phonophoresis[9] have been used to increase the level of drug permeation through the skin. Among them, the use of a penetration enhancer is one of the most convenient methods and has shown relatively good effects.

In a previous report,[10] the release of furosemide from the EVA matrices containing various plasticizers was measured, and the furosemide-EVA matrix system containing the best plasticizer was formulated. To increase the skin permeation of furosemide,[11] various penetration enhancers such as the fatty acids (saturated, unsaturated), the pyrrolidones, the propylene glycol derivatives, the glycerides and the non-ionic surfactants were added to the EVA matrix system, and the level of furosemide permeation through rat skin was evaluated.

The purpose of this study was to develop the new furosemide-EVA matrix formulations containing a permeation enhancer that can provide enhanced transdermal delivery of furosemide for an extended period of time through skin and avoid the side effects.

Materials and Methods

Materials

Furosemide was kindly supplied by Il-yang Pharm. Co., Ltd. (Korea). Ethylene-vinyl acetate (40% vinyl acetate content), myristic acid, linoleic acid, polyoxyethylene-2-stearyl ether (Brij 35), polyoxyethylene-2-oleyl ether (Brij 92), and polyoxyethylene-23-lauryl ether (Brij 72) were purchased from Sigma-Aldrich Chemical Co., Inc. (USA). Lauric acid, oleic acid and caprylic acid were obtained from Tokyo Kasei Kogyo Co. (Japan). 1-methyl-2-pyrrolidone and 2-pyrrolidone were purchased from Acros Organics (USA). The methanol was of HPLC grade. All other chemicals were of reagent grade and used as received.

HPLC determination of furosemide

Furosemide was assayed by HPLC. The mobile phase was a

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combination of 0.01M KH_2PO_4 : methanol (63:37). The column used was a RESTEK C_{18} column (250 × 4.6 mm, 5 μm) and the column temperature was maintained at ambient temperature. A flow rate of 1.5 mL/min yielded an operation pressure of ~1000 psi. The UV detector was operated at a wavelength of 274 nm. Under these conditions, the furosemide peak appeared at a retention time of 7.4 min.

Preparation of furosemide-EVA matrix containing an enhancer

The EVA matrix containing 1.5% furosemide and 10% enhancer was prepared using a solvent casting process described elsewhere.[10] Briefly, the required mass of EVA copolymer beads, the drug and enhancer were dissolved in 20 mL of chloroform. This matrix was poured onto a glass plate and the solvent was allowed to evaporate off at room temperature overnight. The matrix was then removed from the plate and a piece of the matrix was cut from the matrix and the thickness was measured before the experiment. The drug content was calculated from the weight ratio of the drug, enhancer and copolymer used.

Skin preparation

Male rats (ICR strain) were sacrificed in a CO_2 chamber immediately before the experiments. The hair of the abdominal area was carefully removed using electric clippers. A square section of the abdominal skin was excised. After the incision, the adhering fat and other visceral debris in the skin were carefully removed from the undersurface with tweezers. The excised skin was used immediately.[12]

***In vitro* permeation of furosemide from the EVA matrix through the excised skin**

The freshly excised full-thickness skin sample was mounted on the receptor site of the diffusion cell with the stratum corneum side facing towards the donor compartment and the dermal side facing towards the receptor compartment.

The *in vitro* permeation of furosemide from the EVA matrix through the rat skin was examined over a 24-hour period using a modified Franz diffusion cell. An appropriate portion of the matrix was placed on the stratum corneum side, which was then covered with a round glass plate and clamped between the cell cap and the receptor compartment. The diameter of the cell was 1.5 cm, providing an effective constant area of 1.77 cm^2. A 40% (v/v) PEG 400-saline solution was used as the receptor solution, which was maintained at 37°C with a circulating water jacket and stirred at 120 rpm to minimize the boundary effect. At set times, the whole solution was taken from the receptor cell and replaced with an equal volume of fresh medium. The amount of furosemide released from the matrix containing the various enhancers was determined by HPLC at 274 nm. Each data point represents an average of three determinations.

Effects of an enhancer on the permeation of furosemide from the EVA matrix through the rat skin

A furosemide-EVA matrix containing each enhancer was prepared and the level of drug permeation through the rat skin was studied. The enhancers used were non-ionic surfactants such as polyoxyethylene-23-lauryl ether, polyoxyethylene-2-stearyl ether, polyoxyethylene-2-oleyl ether, and glycerides such as oleyl macrogol-6 glycerides, caprylocaproyl macrogol-8 glycerides.

Propylene glycol derivatives, such as propylene glycol monocaprylate, propylene glycol laurate, propylene glycol monolaurate, pyrrolidones such as 2-pyrrolidone, N-methyl-2-pyrrolidone, saturated fatty acids such as linoleic acid, myristic acid, lauric acid, and caprylic acid, and unsaturated fatty acids such as oleic acid, linoleic acid were used.

Calculations

The cumulative amount of furosemide through the rat skin was plotted against time. A linear profile was observed over the 24 hr period and the slope of the linear portion of the curve was determined by linear regression.

The enhancer might affect the lipid fluidity of the stratum corneum structure, which can allow the drug to permeate easily through the rat skin. Generally a penetration enhancer increases the amount of drug released. The effectiveness of the penetration enhancers was defined by the enhancement factor (EF)[13-15] as follows:

EF = (flux of EVA matrix containing enhancer) / (flux of control sample)

Results and Discussion

Effects of an enhancer on the permeation of furosemide through the rat skin

The effects of enhancers such as the non-ionic surfactants, the glycerides, the propylene glycol derivatives, the fatty acids (saturated, unsaturated) and the pyrrolidones on the transport of furosemide through the skin were investigated at an enhancer concentration of 10%.

Fig. 1 shows the permeation profile of furosemide from the EVA matrix containing non-ionic surfactants such as poly-

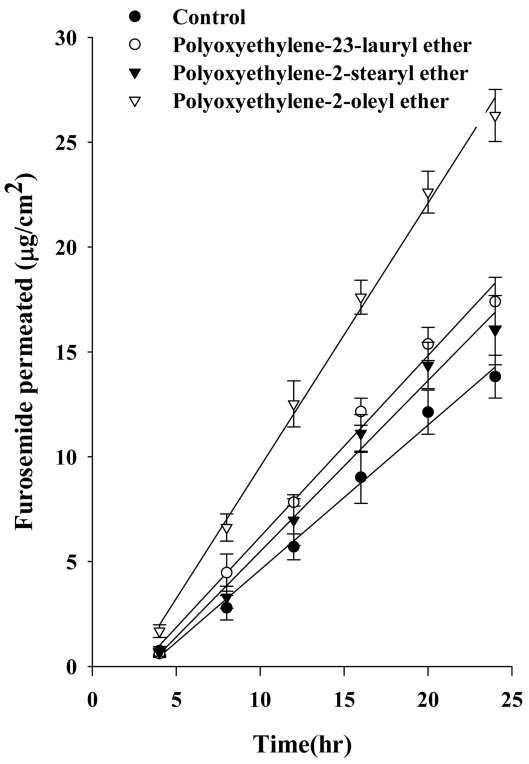

Figure 1–Effects of the non-ionic surfactants on the permeation of furosemide from the EVA matrix through the excised rat skin.

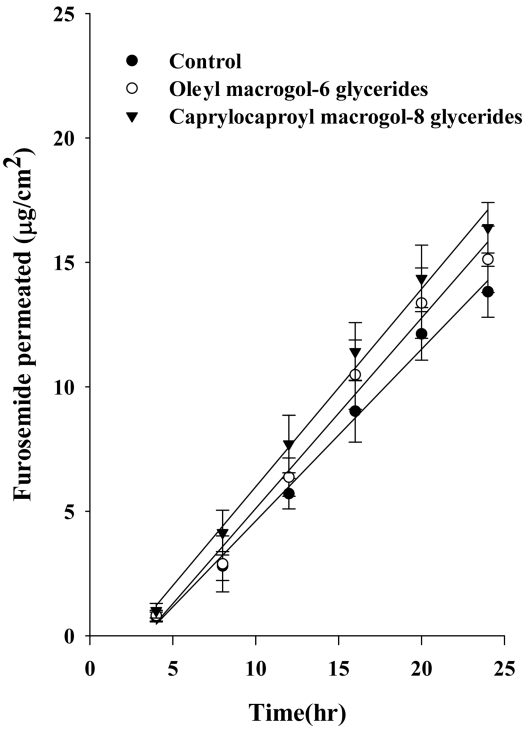

Figure 2–Effects of the glycerides on the level of furosemide permeation from the EVA matrix through the excised rat skin.

Table I–*Effects of the Enhancers on the Level of Drug Permeation from the Furosemide-EVA Matrix through the Rat Skin*

Enhancer	Flux (μg/cm^2/hr)	EF
Control	0.69 ± 0.06	1
Polyoxyethylene-23-lauryl ether	0.87 ± 0.07	1.26
Polyoxyethylene-2-stearyl ether	0.82 ± 0.07	1.19
Polyoxyethylene-2-oleyl ether	1.26 ± 0.05	1.83
Oleic acid	0.78 ± 0.01	1.13
Linoleic acid	0.77 ± 0.03	1.12
Myristic acid	0.83 ± 0.01	1.20
Lauric acid	0.74 ± 0.10	1.07
Caprylic acid	0.77 ± 0.07	1.12
Oleyl macrogol-6 glycerides	0.77 ± 0.06	1.12
Caprylocaproyl macrogol-8 glycerides	0.80 ± 0.05	1.16
Propylene glycol monocaprylate	0.77 ± 0.16	1.12
Propylene glycol laurate	0.71 ± 0.01	1.03
Propylene glycol monolaurate	0.78 ± 0.04	1.13
N-methyl-2-pyrrolidone	0.85 ± 0.07	1.23
2-pyrrolidone	0.76 ± 0.08	1.10

oxyethylene-23-lauryl ether, polyoxyethylene-2-stearyl ether, and polyoxyethylene-2-oleyl ether comparing with the matrix without the enhancers as a control. Like other experiments performed in this laboratory,[14] Brij 92 (polyoxyethylene-2-oleyl ether) had the most enhancing effect. Brij 35 (polyoxyethylene-2-stearyl ether) and Brij 72 (polyoxyethylene -23-lauryl ether) increased the drug permeation rate to some extent. The level of drug permeation from the EVA matrix containing glycerides as the enhancer was measured (Fig. 2). Among the glycerides, caprylocaproyl macrogol-8 glycerides produced a significant permeation rate of furosemide.

The permeation profile of furosemide from the EVA matrix containing the fatty acids showed that the saturated fatty acid group produced a higher permeation rate than the unsaturated fatty acid. Caprylic acid was the most effective among the saturated fatty acids (Table I). The level of furosemide permeation was higher in the matrix containing the pyrrolidones as the enhancer such as 2-pyrrolidone and N-methyl-2-pyrrolidone than the control.

Penetration enhancers are believed to interact with some components of the skin causing increased fluidity in the intercellular lipid lamellae, swelling and/or leaching out of some of the structural components from the stratum corneum and thus increase the level of drug penetration through the barrier membrane.[16-19]

Many penetration enhancers have a molecular structure and charge density that is suitable for interacting with the polar head groups of the lipids. Therefore, they may disturb the hydrated regions and head group interactions as well as fluidize these regions, thereby increasing the penetration of polar (hydrophilic) compounds. A more aqueous fluid may enter the tissue, increasing the interlamellar volume and thus the volume available for polar diffusion. The enhancer might also affect the lipid fluidity of the stratum corneum structure, which can allow the drug to permeate more easily through the rat skin.

The permeation enhancing effects were evaluated using the enhancement factor (Table I). The level of drug permeation from EVA matrix containing the enhancer through the rat skin was higher than the control. Among the enhancers used in these tests, Brij 92 (polyoxyethylene-2-oleyl ether) had the largest effect, showing a 1.83 fold increase in permeability. An EVA matrix containing polyoxyethylene-2-oleyl ether as a permeation enhancer could be developed for the enhanced transdermal delivery of furosemide.

Conclusions

The rate of furosemide release was higher in the EVA matrix containing the enhancer than with the control. Among the various enhancers used, such as non-ionic surfactants, glycerides, propylene glycol derivatives, fatty acids (saturated, unsaturated), pyrrolidones, polyoxyethylene-2-oleyl ether showed the most enhancing effects. The polyoxyethylene 2-oleyl ether as a permeation enhancer could be used for development of furosemide-EVA transdermal matrix system.

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