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Enhancement of percutaneous absorption of naproxen by phospholipids

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Abstract

The skin penetration of naproxen from various phospholipid gel formulations through human cadaver skin was investigated in diffusion chambers. Presence of phospholipids decreased the skin penetration of naproxen from aqueous gels. The addition of 32% (m/m) ethanol or propylene glycol in the aqueous gel formulation, with the presence of phospholipids, apparently increased the percutaneous absorption of naproxen. The penetration enhancement effect of phospholipid with ethanol was, however, more significant than that of phospholipid with propylene glycol. The effect of ethanol concentration on the ability of phospholipids to increase penetration of naproxen, was evaluated in pretreatment studies. The results showed that more than 8% (m/v) ethanol is needed for the enhancing effect of phospholipids. The concentration of phospholipid and the presence of unsaturated fatty acids in phospholipids are also important factors affecting the transdermal flux of naproxen. In conclusion, in the presence of cosolvent, phospholipids increase the transdermal flux of naproxen.

Keywords: Ethanol; Gel; Naproxen; Percutaneous absorption; Phospholipid

1. Introduction

Topical application of drugs has been studied widely in recent years but poor permeability of most drugs through the skin limits the use of topical drug administration. Drug penetration can in some cases be increased with enhancers which efficiently decrease the barrier resistance of the stratum corneum (Barry, 1987). The main problem of penetration enhancers is skin irritation.

Phospholipids are a potential group of penetration enhancers. Being composed of natural body constituents and being biodegradable (Storm et

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Table 1
Compositions of gel formulations

Constituents	Amount, % m/m, in the Formulations					
	I	II	III	IV	V	VI
Naproxen	5	5	5	5	5	5
Carbopol 934	2	2	2	2	2	2
Ethanol	32	32	0	0	0	0
Water	47	43–46.96	79	77.8–78.6	47	45.8–46.6
Propylene glycol	0	0	0	0	32	32
Phospholipid	0	0.04–4.0	0	0.4–1.2	0	0.4–1.2
Other	14	14	14	14	14	14
TOTAL	100	100	100	100	100	100

al., 1991), topically administered phospholipids can be generally considered as safe. Although the behaviour of phospholipids has been investigated in numerous studies (Ganesan et al., 1984; Foldvari et al., 1990; Kirjavainen et al., 1996; Yokomizo and Sagitani, 1996a), the exact mechanism is not fully understood and the results are still somewhat contradictory.

Naproxen is a potent nonsteroidal anti-inflammatory drug (NSAID) used for a variety of inflammatory conditions (Singh and Roberts, 1994). Like other NSAIDs, the most common side effect of peroral naproxen is gastrointestinal irritation. Thus, the possibility of delivering naproxen through the skin for local inflammations at low doses is attractive. Penetration enhancers are needed because otherwise only small amounts of naproxen passes through the skin (Van den Ouweland et al., 1989).

The aim of this study was to determine the in vitro skin penetration of naproxen from different, phospholipid containing gel formulations, in order to find out the importance of cosolvents, such as ethanol and propylene glycol, on skin permeation of naproxen from the gels.

2. Materials and methods

2.1. Materials

L-α-phosphatidylcholine (EPC, 60%, from fresh frozen egg yolk) and L-α-phosphatidylcholine (SPC, 60%, from soybean) were purchased from Sigma (St. Louis, MO, USA). The model drug was naproxen (Orion Corporation, Finland). The Carbopol 934 was from BF-Goodrich (Belgium), propylene glycol from Merck (Darmstadt, Germany) and the ethanol from Primalco Oy (Rajamäki, Finland). All components were of pharmacopoeia quality. All other chemicals used were at least reagent grade.

Skin samples were obtained from the abdominal area of female cadavers from Kuopio University Hospital (Kuopio, Finland) and Turku University Hospital (Turku, Finland). The skin samples were immersed in distilled water at 60°C for 2 min and the stratum corneum was then removed from the epidermis using a pair of tweezers. Skin samples were cut into pieces, dried and frozen prior to use.

2.2. Compositions of gels

Gel formulations containing 5% naproxen were prepared. Various gel formulations are listed in Table 1. Formulations I and II were hydroalcoholic gels, Formulations III and IV were aqueous gels and Formulations V and VI were propylene glycol containing gels. The phospholipid concentration of the Formulation II, IV and VI varied from 0.04 to 4.0% (m/m). The phospholipids used were EPC and SPC.

2.3. Permeation studies

The permeation studies were carried out using the Franz-type diffusion cell (Crown Glass Company, Somerville, NJ, USA). Samples of dried skin were rehydrated by immersion in phosphate buffered saline (PBS) pH 7.4 at room temperature for 15 min before being mounted in the diffusion cell. The exposed skin surface area was 1.77 cm^2 or 0.64 cm^2 and the receptor volume was 12 ml or 5 ml. The receptor compartment was filled by PBS which was stirred and kept at 37°C during the experiments. A sample of 0.5 g of 5% naproxen gel was administered onto the stratum corneum side of the skin mounted into the chamber. The donor compartment was covered with cellophane and Parafilm. The control gel was the 5% naproxen gel without phospholipids. The receptor compartment was sampled (500 μl) at the appropriate time and replaced by an equal volume of fresh PBS. The drug concentrations of the samples were determined by high-performance liquid chromatography (HPLC). The results represent the mean ± S.E.M. (n varies from 3 to 9) including skin from one to three different donors.

2.4. Pretreatment studies

The upper side of skin was treated with EPC in an ethanol solution for 24 h at 37°C in the Franz diffusion chambers. The concentration of the EPC was 0.4% (m/m) and the concentration of ethanol was 32% or 8% (m/v). The control skin was treated with a corresponding concentration of ethanol without EPC. After 24 h, pretreatment solutions were removed and 0.5 g of 5% naproxen gel was applied to the skin and the study was carried out as described above. The results represent the mean ± S.E.M. ($n = 3$).

2.5. HPLC assay

To determine the concentrations of naproxen, a Hewlett-Packard 1050 HPLC system equipped with a UV detector (HP 1050) at 270 nm was used in reversed-phase mode with a Hypersil BDS C18 column (3 μm, 4.0 × 100 mm, Shandon Scientific, England). The mixture of 0.02 M phosphate buffer pH 3.0 and acetonitrile (50:50) was employed as the mobile phase at a flow rate of 1 ml/min. The retention time was 2.4 min.

2.6. Calculation of results

The cumulative amount of naproxen penetrating the skin was plotted against time. Drug flux (μg/cm^2 per 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 diffusion took place. The permeabilities, K_p (cm/h) of the naproxen were calculated by dividing the drug fluxes by the initial concentration of the drug in the donor phase.

3. Results

3.1. Percutaneous penetration of naproxen from hydroalcoholic gel

The skin permeation of naproxen from EPC containing gels (Formulation II) is summarized in Fig. 1. The results show that 0.4% EPC and 4% EPC were able to increase the permeation of naproxen. In contrast, 0.04% EPC did not seem

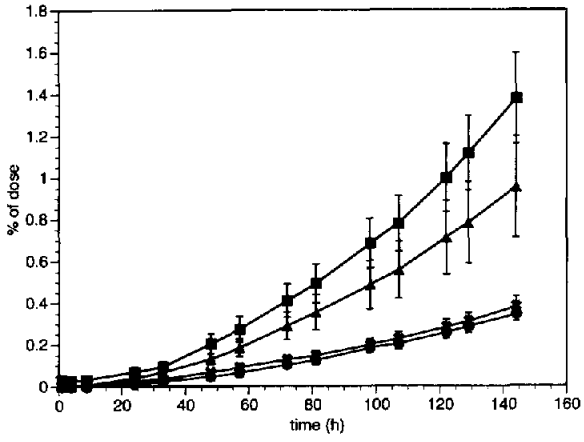

Fig. 1. The amount (% of dose, mean ± S.E.M., $n = 3$–9) of naproxen penetrated across human epidermis in vitro after topical application of 5% naproxen in hydroalcoholic gels (Formulation II); containing 0.04% EPC (●); containing 0.4% EPC (▲); and containing 4% EPC (■). The control (Formulation I) (×) contained no EPC.

Table 2
The permeability coefficients (K_p) of naproxen in human skin in vitro (mean ± S.E.M., $n = 3$–6)[a]

	Formulation[b]	K_p (10^{-5} cm/h)
Hydroalcoholic gels		
Control	I	1.5 ± 0.3
0.4% SPC	II	5.2 ± 1.6
Aqueous gels		
Control	III	10.7 ± 2.5
0.4% SPC	IV	3.4 ± 0.8
1.2% SPC	IV	3.7 ± 2.7
Propylene glycol gels		
Control	V	1.9 ± 0.2
0.4% SPC	VI	3.6 ± 1.2
1.2% SPC	VI	4.8 ± 2.6

[a] The studies were carried out as described in Section 2.3. The skin was from one to three different donors and the skin of the same donors were used in the control and in the phospholipid containing studies.
[b] The codes for gel formulations and their composition is described in Table 1.

to have a notable effect. When comparing to the control (Formulation I), the flux of naproxen from 0.4% EPC and 4% EPC containing gels increased 2- and 4-fold, respectively.

The permeabilities of naproxen from SPC containing gel (Formulation II) is presented in Table 2. 0.4% SPC containing gel caused a significant increase of penetration of naproxen as compared to the control (Formulation I), the naproxen flux increased 3.5-fold.

Table 3
In vitro the permeation coefficients (K_p) of naproxen after pretreatment with phospholipid-ethanol solutions in the human skin (mean ± S.E.M., $n = 3$)[a]

Pretreatment	K_p (10^{-4} cm/h)
Control	2.3 ± 0.2
32% ethanol +0.4% EPC	6.5 ± 1.4
Control	0.6 ± 0.1
8% ethanol +0.4% EPC	0.1 ± 0.1

[a] The studies were carried out as described in Section 2.3. The skin of the same donors were used in the control and in the phospholipid containing studies.

3.2. Percutaneous penetration of naproxen from aqueous gel

The skin permeabilities of naproxen from aqueous gels (Formulations III and IV) are summarized in Table 2. According to the result of the studies the SPC does not enhance, but actually decreases skin permeation of naproxen. The concentration of phospholipid did not affect the permeation of naproxen in the skin.

3.3. Percutaneous penetration of naproxen from propylene glycol containing gel

Table 2 shows the rate of penetration of naproxen from propylene glycol containing gels (Formulations V and VI). Naproxen flux was increased with 0.4 and 1.2% SPC, but enhancement was not as significant as in the presence of ethanol. The flux of naproxen increased 1.9- and 2.5-fold, respectively.

3.4. Pretreatment studies

The rate of penetration of naproxen after the pretreatment with phospholipid–ethanol solutions is presented in Table 3. As can be seen, EPC in the 32% ethanol solution increased skin permeation of naproxen, compared to the control solution. Thus, this concentration of ethanol had a synergic effect on the ability of phospholipids to promote skin permeation of naproxen. In contrast, 8% ethanol did not display a synergic effect, skin pretreatment with EPC in the 8% ethanol solution decreased the permeation of naproxen as compared to the control. This pattern corresponds to the percutaneous penetration of naproxen from aqueous gel, the phospholipid was not able to affect percutaneous penetration of naproxen without ethanol.

4. Discussion

In the penetration studies the phospholipid gels were compared to the formulations without phospholipids. The results indicated that both EPC and SPC are effective skin penetration enhancers

of naproxen when applied in hydroalcoholic or propylene glycol gels. Many groups have reported that EPC or SPC dissolved in propylene glycol enhance the transdermal flux of several drugs (Kato et al., 1987; Kimura et al., 1989; Mahjour et al., 1990). Both EPC and SPC contain phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and unsaturated fatty acids. The presence of unsaturated fatty acids in the phospholipids may be responsible for the enhancement (Yokomizo, 1996; Yokomizo and Sagitani, 1996b). Unsaturated fatty acids have very different lipid-packing properties compared to saturated fatty acids due to a 'kink' in the *cis*-alkenyl chain. The packing nature of unsaturated fatty acids disrupts the stratum corneum lipid structure and enhances the percutaneous penetration of drugs. Futhermore, Yokomizo and Sagitani (1996c) have reported that the phospholipids containing unsaturated fatty acids strongly raising the fluidity of the stratum corneum due to its having a low value 'transition temperature' (T_m). EPC and SPC having an unsaturated acyl chain exist as fluid at skin temperature.

In our studies the penetration of naproxen was increased by increasing the amount of phospholipid. However, there were no more significant differences among 0.4 and 4% EPC containing gels. Kimura et al. (1989) found that the enhancing effect on flufenamic acid was lost when the phosphatidylcholine content exceeded 40 μmol/ml. The same effect was also reported by Nishihata's group (Nishihata et al., 1987), contents of phospholipid above 0.5% (m/v) did not further increase diclofenac penetration. It seems that the amount of phospholipid to get the maximum penetration of the drug, may be dependent on the characteristics of the drug. It has been reported that percutaneous penetration of a water soluble drug is not dependent on the phospholipid concentration, while in contrast, percutaneous penetration of a lipophilic drug was proportional to the concentration of phospholipid (Yokomizo, 1996).

Pretreatment with the phospholipid in the ethanol solution was performed to clarify whether phospholipids carried the naproxen from the gel to the skin surface or whether the phospholipids affected on the structure of skin. Furthermore, we wanted to find out the importance of the ethanol concentration in the phospholipid gel formulation. The results suggest that phospholipids affect the structure of the skin by reducing the skin resistance to penetration of naproxen and that the presence of a certain amount of ethanol is necessary for the enhancing effect of phospholipids. In a previous study, the influence of ethanol on the skin penetration of the different liposomes was investigated by confocal laser scanning microscopy (CLSM) (Kirjavainen et al., 1998). The results showed that when the liposome suspension contained 32% ethanol, a fluorescent lipid probe of EPC penetrated deeply into the stratum corneum. In contrast, without ethanol there was only a slight fluorescent band on the surface on the skin. In the same study, the percutaneous penetration of different drugs from the solution in the presence of EPC and 32% ethanol was also investigated. The penetration of drugs was significantly increased compared to the control solution, containing a 32% ethanol–drug solution without EPC. It seems that ethanol affects in two ways. First, ethanol raises fluidity of skin lipid multilayers so that phospholipid can penetrate into the skin. Secondly, ethanol loosens phospholipid vesicles so that phospholipids may penetrate into the skin and disrupt the bilayer structure of the stratum corneum.

Penetration of naproxen from aqueous, hydroalcoholic and propylene glycol containing gels was compared. The penetration enhancing effect of phospholipid was observed in ethanol and propylene glycol containing gels. Propylene glycol does not affect the lipid structure of skin like ethanol does. It works as an enhancer by dissolving alphakeratin and by occupying hydrogen-bonding sites, thus reducing drug/protein tissue binding (Barry, 1987). Propylene glycol is often used with other enhancers, because it considerably increases the efficacy of enhancers that affect the lipids in the stratum corneum. This is consistent with our studies if we suppose that SPC works as an enhancer by affecting lipid structure of stratum corneum and propylene glycol increased the ability of SPC to promote skin permeation of naproxen. However, propylene glycol did not dis-

play as significant a synergic effect with phospholipids as ethanol did. This is logical because propylene glycol has a different site of action in the skin compared to ethanol and phospholipids. Other explanations may be the different solubilities of phospholipids in propylene glycol and ethanol. Yokomizo and Sagitani (1996a) reported that phospholipids which had the best enhancer effect on penetration of indomethacin also had the best solubility in propylene glycol.

The presence of phospholipids decreased skin permeation of naproxen from aqueous gel. It is plausible that SPC is not able to penetrate into the skin from aqueous gel and thus it forms an extra barrier onto the skin surface. The effect of water on skin permeation of the drug is usually low. Water works as a penetration enhancer by loosening the lipid packing in the skin by associating via hydrogen-bonding with lipid polar head groups (Barry, 1987). In addition, it binds to the protein chains, thus reducing interaction between them.

In conclusion, the present results show that EPC and SPC are not able to increase the skin penetration of naproxen from aqueous gels, but the addition of cosolvents, such as ethanol or propylene glycol into the formulations, increased the percutaneous penetration of naproxen.

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