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# Effectiveness and Mode of Action of Isopropyl Myristate as a Permeation Enhancer for Naproxen through Shed Snake Skin

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## Abstract

The effectiveness and mode of action of isopropyl myristate (IPM) as an enhancer for the permeation of naproxen through shed snake skin have been investigated.

The highest naproxen permeability was afforded by IPM ($36{\cdot}2 \times 10^{-4}$ cm $h^{-1}$), followed by menthol ($25{\cdot}0 \times 10^{-4}$ cm $h^{-1}$), oleic acid ($11{\cdot}1 \times 10^{-4}$ cm $h^{-1}$), azone ($7{\cdot}3 \times 10^{-4}$ cm $h^{-1}$) and control ($1{\cdot}4 \times 10^{-4}$ cm $h^{-1}$). Whereas the permeability of un-ionized naproxen ($47{\cdot}4 \times 10^{-5}$ cm $h^{-1}$) was much greater than that of ionized naproxen ($1{\cdot}11 \times 10^{-5}$ cm $h^{-1}$), IPM-treatment of the intact skin increased the flux of ionized naproxen significantly more (50-fold) than that of un-ionized naproxen (15-fold). The large effect of pH on the permeation of naproxen through the intact stratum corneum became insignificant after extraction of lipids from the skin. Similar permeation of naproxen through intact and delipidized skin after IPM treatment indicated that the lipid barrier of the skin was largely impaired by IPM. Direct application of IPM to skin yielded a 2·6-fold higher naproxen permeability than the application of IPM as a gel. A greater amount of naproxen was absorbed from 1% test gel (pH 5) containing IPM than from 10% commercial gel (pH 7) containing no IPM.

These results show that use of IPM can significantly improve the bioavailability of naproxen in topical preparations.

Non-steroidal anti-inflammatory drugs (NSAIDs) are most commonly administered orally, despite a high incidence of gastrointestinal adverse effects, particularly after extended use. Because of their potential adverse effects, an alternative route has been sought for administration of NSAIDs. The primary benefit of topical delivery of NSAIDs is the direct accessibility of the drugs to the target site with minimal systemic side effects.

Because of the strong barrier function of the stratum corneum, a small number of drugs permeate the skin in therapeutic quantities and the efficacy of various absorption enhancers has been actively investigated in recent years. Isopropyl myristate (IPM) has been widely used as a vehicle in many cosmetic and pharmaceutical preparations because of its emollient properties, its safety and its compatibility with a wide range of compounds.

Shed snake skin has been used as a model membrane for studying the effects of different enhancers and other formulation variables on the percutaneous absorption of naproxen. Shed snake skin is a non-living, pure stratum corneum with no hair follicles. The mesos layer, the main permeation barrier, is primarily composed of keratin-filled corneocytes and intercellular lipid. Its advantages and limitations as a model membrane have been discussed previously (Higuchi & Konishi 1987; Itoh et al 1990). Some of the benefits include similar thickness, lipid composition and barrier function resembling the stratum corneum in man, and multiple skin samples are available from a single snake.

In this study, the effectiveness of several compounds as enhancers of skin permeation for naproxen was determined in-vitro. A further study was conducted to assess the efficacy and mode of action of IPM for the transport of naproxen through shed snake skin under various conditions.

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## Materials and Methods

### *Materials*

The enhancers tested were isopropyl myristate (Sigma Chemical Co., St Louis, MO, USA), l-menthol (Amend Drug & Chemical Co., Irvington, NJ, USA), oleic acid (Fisher Scientific Co., Fair Lawn, NJ, USA), and azone (Discovery Therapeutics Inc., Richmond, VA, USA). All chemicals were reagent grade and used as received.

### *Gel preparation*

Pluronic gels containing 1% naproxen were prepared by the cold method described by Schmolka (1972); PF-127 (25% w/w) was slowly added to phosphate buffer (0·2 M; pH 5) in a 50-mL beaker and left in a refrigerator (4°C) overnight to complete the dissolution of the polymer. After the formation of a clear and viscous solution in ethanol naproxen was added to the cold PF-127 solution (5–10°C) and thoroughly mixed. The solution was left at room temperature for approximately 30 min until a clear gel was formed.

### *In-vitro permeation test*

Shed skins from the snake *Elaphe obsoleta*, from Sandy Creek Nature Center (Athens, GA, USA), were used for in-vitro permeation studies. The pieces of skin (10 $cm^2$), after soaking in water for 30 min for cleansing and easy handling, were mounted on Franz diffusion cells. The skins were then left to equilibrate for 2 h in the system with circulating water (32°C) and their thickness was measured using a thickness gauge (Teclock, Teclock Corporation, Chicago, IL). The gel (0·5 g) was uniformly applied to the 2-$cm^2$ diffusion area of the skin,

and the donor chamber was covered with parafilm. The receptor chamber was filled with phosphate buffer (0·2 M, pH 7·4; 6 mL) and continuously stirred at 800 rev $min^{-1}$ with a magnetic bar. At scheduled time intervals, portions of the receptor phase (50 μL) were withdrawn for the quantitation of naproxen by the HPLC method reported by Irwin et al (1990) with minor modification. The receptor phase was replenished with fresh phosphate buffer (pH 7·4; 50 μL) after each sampling.

*Pretreatment of skin with enhancer*

After equilibrating the skin for 1 h in the diffusion cell at 32°C, enhancer solution (150 μL) was applied evenly to the skin using a micro-pipette. After 1 h the 1% naproxen gel (0·5 g) was spread evenly on the skin, and the donor chamber was covered with parafilm. The enhancers tested were 10% isopropyl myristate in *N,N*-dimethylformamide (IPM-DMF), 10% oleic acid in propylene glycol (OA-PG), 5% azone in propylene glycol (AZ-PG) and 5% 1-menthol in 50% ethanol (MT-50% EtOH). The effect of solvents (PG, 50% ethanol and DMF) on the permeation of naproxen through the skin was also determined.

*Skin delipidization*

Pieces of dry shed snake skin were individually weighed and placed in a 50-mL glass bottle containing chloroform-methanol (2:1, v/v; 30 mL) which was gently agitated for 24 h at room temperature using a shaker (Junior Orbit Shaker, Lab-Line Instruments Inc., Melrose Park, IL, USA). Skins were removed, rinsed twice with fresh chloroform-methanol mixture and dried in a desiccator to constant weight. The lipid content of the skin, determined as the change in weight after solvent extraction, was 6·2 ± 0·65% (mean ± s.d., n = 9) of the total weight; this was in close agreement with the value of 5·9% found by Roberts & Lillywhite (1983) for shed skin of the same species.

## Results and Discussion

The transport of drug molecules from a topical vehicle across the skin is primarily governed by the vehicle-membrane partition coefficient of drug and the rate at which the partitioned molecules diffuse through the membrane. On the basis of the quasi-steady-state condition which occurred in the in-vitro diffusion study, permeation parameters such as the flux, partition coefficient and diffusivity were determined and used to assess the effectiveness of the enhancers.

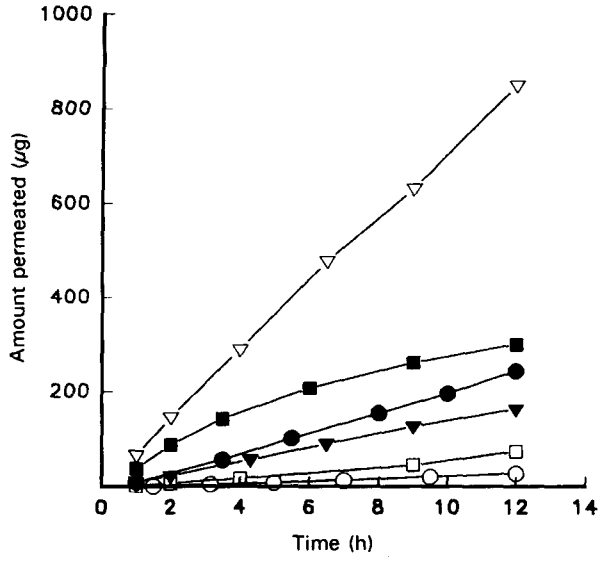


FIG. 1. Effect of various enhancers on the permeation of naproxen through shed snake skin: ○ control; □ dimethylformamide; ▼ azone in propylene glycol; ● oleic acid in propylene glycol; ■ menthol in ethanol; ▽ IPM in dimethylformamide.

*Effect of enhancers*

Fig. 1 shows the time-courses of cumulative amounts of naproxen permeating through shed snake skin pre-treated with different enhancers. The plots obtained typically showed an initial lag-time followed by a linear period consistent with the process of steady-state Fickian diffusion. Regression analysis of the steady-state portion of the plots enabled determination of the flux and lag-time; these are presented in Table 1. The effectiveness of a permeation enhancer is often measured after pre-treating the skin with the enhancer before applying the medication. This method of application enables direct assessment of enhancer activity without the results being affected by the formulation. It was found that enhancer-treated skins increased the diffusion flux of naproxen at least fivefold while reducing the lag-times from hours to minutes. Other parameters, diffusion and partition coefficients of naproxen, were determined using the equations:

$$J_S = (\Delta Q/\Delta t)_S/A = P \times C_d = D \times K \times C_d/h \quad (1)$$

$$D = h^2/6L \quad (2)$$

where $J_S$ is the permeation flux (μg $cm^{-2}$ $h^{-1}$) at steady state, A the diffusional surface area ($cm^2$), $(\Delta Q/\Delta t)_S$ the slope of the permeation plot at steady state (μg $h^{-1}$), P the permeability coefficient (cm $h^{-1}$); $C_d$ the drug concentration in the donor

Table 1. Permeation parameters of naproxen obtained before and after treatment of the skin with various enhancers.

| | P ± SD (cm $h^{-1}$) × $10^3$ | L[a] ± SD (h) | D ($cm^2$ $h^{-1}$) × $10^6$ | K | ER[b] |
|---|---|---|---|---|---|
| Control | 0·14 ± 0·07 | 2·27 ± 0·13 | 0·47 | 0·77 | – |
| 10% OA-PG | 1·06 ± 0·09 | 0·71 ± 0·06 | 1·52 | 1·78 | 7·57 |
| 5% MT-EtOH | 2·45 ± 0·21 | 0·21 ± 0·02 | 5·02 | 1·24 | 17·5 |
| 10% IPM-DMF | 3·57 ± 0·23 | 0·012 ± 0·001 | 86·35 | 0·11 | 25·5 |
| 5% AZ-PG | 0·73 ± 0·03 | 0·37 ± 0·02 | 2·93 | 0·63 | 5·21 |

[a]Lag-time. [b]Enhancement ratio.

phase ($\mu g\ mL^{-1}$), D the diffusion coefficient ($cm^2\ h^{-1}$), K the partition coefficient between vehicle and skin, h the membrane thickness (cm), and L the lag-time (h). The enhancement ratio (ER) is defined as the quotient of the permeability coefficients obtained after and before treatment with enhancer. As shown in Table 1, IPM-DMF afforded the greatest enhancement ratio (ER = 26), followed by MT-50% EtOH (ER = 17·5), OA-PG (ER = 7·6), and AZ-PG (ER = 5·2). PG ($P = 0{\cdot}17 \times 10^{-3}$ cm $h^{-1}$) and 50% EtOH ($P = 0{\cdot}15 \times 10^{-3}$ cm $h^{-1}$), which were used as solvents for the enhancers, by themselves showed little enhancing effect on naproxen permeability over the control ($P = 0{\cdot}14 \times 10^{-3}$ cm $h^{-1}$). DMF alone, however, approximately doubled the permeability of naproxen ($0{\cdot}33 \times 10^{-3}$ cm $h^{-1}$), but no significant difference was found between the permeabilities of naproxen through skin treated with IPM alone ($3{\cdot}48 \times 10^{-3}$ cm $h^{-1}$) or with IPM-DMF ($3{\cdot}57 \times 10^{-3}$ cm $h^{-1}$).

Since drug transport through the skin usually occurs by passive diffusion, the analysis of permeation data using Fick's first law offers useful information about enhancer activity. It was found that the permeation of naproxen through shed snake skin increased, in the presence of enhancers, primarily as a result of increased diffusion coefficients of naproxen as shown in Table 1. No clear correlation between the partition coefficient and permeability of naproxen was, however, observed in this study. Whereas OA-PG and MT-50% EtOH significantly enhanced the gel-skin partition coefficients of naproxen, IPM-DMF and AZ-PG had the opposite effect (Table 1). The decreased partition of naproxen into the skin in the presence of IPM-DMF and AZ-PG might be explained on the basis of the modified form of equation 1:

$$dQ/dt = a_v/\gamma_S \cdot (A \cdot D/h)$$

where $a_v$ is the thermodynamic activity of drug in the vehicle and $\gamma_S$ is the effective activity coefficient of the drug in the skin barrier (Higuchi 1960). In the permeation study employing skin pre-treated with an enhancer, the diffusion coefficient (D) and the activity coefficient ($\gamma_S$) of the drug are subjected to change as a result of the presence of the enhancer in the skin. Considering that the thermodynamic activity of naproxen in the vehicle remained the same, the reduced partition coefficient after IPM-treatment compared with the control could be because of the increased activity coefficient (reduced solubility) of naproxen in IPM-treated skin. It is, therefore, concluded that IPM exerted its enhancing action on the permeation of naproxen through the snake skin primarily by increasing the diffusion coefficient rather than the partition coefficient of drug in the membrane. The greater diffusion coefficient of naproxen in the presence of IPM in the skin could be because of the reduced solvation of naproxen molecules in the more lipophilic environment created by IPM.

*Effect of delipidization*

If the intercellular lipids in the stratum corneum constitute the primary barrier function of the skin, the removal of these lipids should increase the skin permeability of drugs. Scheuplein & Ross (1970) reported that extraction of lipids from the stratum corneum in man resulted in: a significant increase in skin permeability to both polar propanol and non-polar heptanol; similar permeability to both these compounds; and reduced activation energy for the diffusion of these compounds.

In this study, the effect of skin delipidization on the permeability of naproxen was investigated at pH 5 and 8. It was found that the extraction of lipids from the stratum corneum using a 2 : 1 (v/v) mixture of chloroform and methanol resulted in 9·7-fold and 153-fold increases in the permeability of naproxen at pH 5 ($1{\cdot}40 \times 10^{-3}$ cm $h^{-1}$) and pH 8 ($1{\cdot}70 \times 10^{-3}$ cm $h^{-1}$), respectively. Consequently, the large effect of pH on the permeability of naproxen through the intact stratum corneum found between pH 5 ($14{\cdot}4 \times 10^{-5}$ cm $h^{-1}$) and pH 8 ($1{\cdot}11 \times 10^{-5}$ cm $h^{-1}$) has dissipated after skin delipidization. Apparently, both the barrier function and selectivity of the stratum corneum for naproxen were highly compromised by the extraction of the lipids in the skin. Similar permeation profiles of naproxen for delipidized ($P = 3{\cdot}61 \times 10^{-3}$ cm $h^{-1}$) and intact ($P = 3{\cdot}57 \times 10^{-3}$ cm $h^{-1}$) skins, both treated with IPM, suggested that IPM highly disrupted the lipid barrier of the skin. The treatment of delipidized skin with IPM increased the permeability of naproxen by 2·6-fold over untreated delipidized skin, however. The further effect of IPM on the delipidized skin suggested that other enhancing mechanisms might be involved in addition to its lipid disrupting action. Because IPM possesses both polar and non-polar characteristics, modification of polar pathways in the stratum corneum by interaction with the intracellular proteins could be one of the mechanisms involved. Enhancer activity of this type was suggested by Barry (1987) for an amphiphilic compound, decylmethylsulphoxide.

*Effect of pH*

Most of the physicochemical basis for the percutaneous absorption of drugs has been developed for non-electrolytes, and only a few of the studies reported in the literature have dealt with the effect of pH on the permeation of ionizable compounds through the skin (Loveday 1961; Arita et al 1970). Evidently, the process of transport of ionizable compounds could be highly complicated because of the presence of both ionized and un-ionized species, each of which might permeate the skin at a considerably different rate.

Naproxen is a weak acid ($pK_a = 4{\cdot}15$) with strong lipophilic character, as is shown by the high log octanol/water partition coefficient of 3·18; the pH of the vehicle will, therefore, affect the overall permeation rate of naproxen. In this study, the effect of pH on the transport of naproxen through shed snake skin was studied over the pH range 2–8. Fig. 2 shows that naproxen permeation rates are much higher at lower pH than at higher pH; this is consistent with the principle of non-ionic diffusion through a biological membrane. Although the permeability of un-ionized naproxen at pH 2 ($47{\cdot}4 \times 10^{-5}$ cm $h^{-1}$) was about 43-fold that of ionized naproxen at pH 8 ($1{\cdot}11 \times 10^{-5}$ cm $h^{-1}$), the relatively high permeation found for ionized naproxen is indicative of the presence of a polar pathway in the skin.

The effects of IPM on the skin permeation of un-ionized and ionized naproxen were investigated at pH 2 and pH 8. IPM-treatment of the intact skin increased the transport of ionized naproxen significantly more (51-fold) than that of un-ionized naproxen (15-fold), indicating a significant reduction of the barrier function of the stratum corneum by IPM, particularly for the ionized form of naproxen.

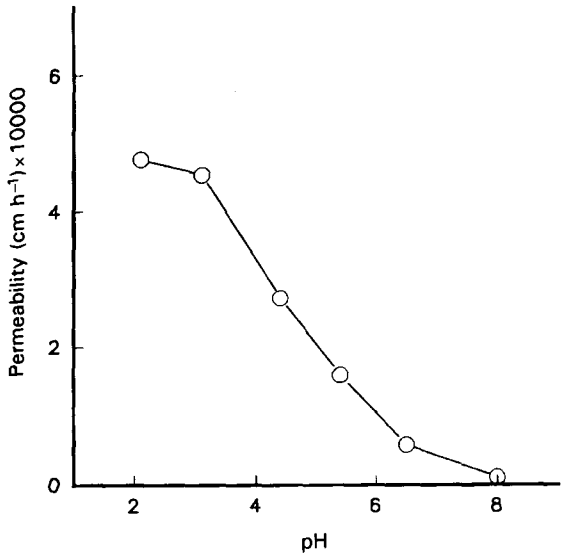


FIG. 2. Effect of formulation pH on the permeation of naproxen through shed snake skin.

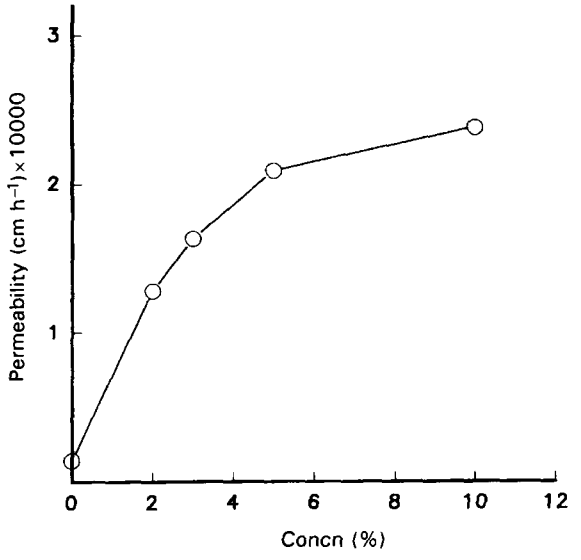


FIG. 3. Effect of IPM concentration in gel on the permeation of naproxen through shed snake skin.

*Effect of formulation*

Fig. 3 shows that for IPM concentrations between 2% and 10%, the increase in naproxen permeation ranged from 8·9- to 16·6-fold compared with the control containing no IPM. Despite a large potential effect of the enhancer on the physicochemical properties of the vehicle and the thermodynamic activity of the drug in the formulation, enhancer activity is often measured using the incorporation method instead of the skin-pretreatment method. It was found that, for the same amount of IPM used, the enhancement ratio of IPM obtained from the incorporation method (ER = 9·5) was much lower than that from the pretreatment method (ER = 25). The smaller enhancement of permeation resulting from the incorporation method could be attributed to the reduced thermodynamic activity of naproxen in the vehicle and the lower amount of IPM available for the skin as compared with the pretreatment method. A similar result was reported by Sheth et al (1986) who observed that the flux of trifluorothymidine through guinea-pig skin pre-treated with azone was significantly higher than that when the vehicle containing azone was applied to the skin.

The effectiveness of IPM as a permeation enhancer was further demonstrated by comparing the percutaneous absorption of naproxen between the test gel containing 1% naproxen and 2% IPM and the commercial gel containing 10% naproxen. As shown in Fig. 4, a greater amount of naproxen was absorbed from the test gel (pH 5) containing IPM than from the commercial gel (pH 7), despite the smaller amount of naproxen in the test gel. Over the period of 24 h, 0·63% and 8·47%, respectively, of the applied doses were absorbed from the commercial gel and the test gel containing IPM. When an equal amount of IPM was added to the 10% commercial gel, however, the total amount of naproxen absorbed from the commercial gel was much greater than from the test gel.

In conclusion, among several absorption enhancers tested, IPM had the highest enhancer effect for the permeation of naproxen through shed snake skin. IPM greatly increased the diffusivity of naproxen through the skin, while decreasing its

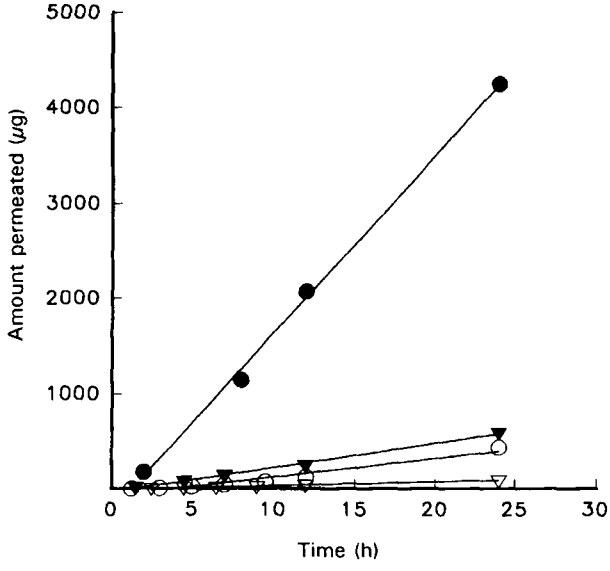


FIG. 4. Effect of IPM on the permeation of naproxen from 1% test gel and 10% commercial gel: ▽ 1% test gel; ○ 10% commercial gel; ▼ 1% test gel + IPM; ● 10% commercial gel + IPM.

partition coefficient between the gel and skin. The enhancer activity of IPM was concentration-dependent over the range of 2–10% and was significantly more pronounced for ionized than for un-ionized naproxen. Permeabilities of ionized and un-ionized forms of naproxen through the delipidized skin were comparable, which suggested that the barrier function and selectivity of the skin were largely impaired by the removal of lipids from the skin. Similar permeation of naproxen through intact and delipidized skins treated with IPM are indicative of the disruptive action of IPM on the skin lipids. The greater amount of naproxen absorbed from the 1% naproxen gel containing IPM than from the 10% naproxen gel without IPM suggests that the use of IPM can improve the therapeutic value of naproxen in topical preparations.

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