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Alkyl esters as skin permeation enhancers for indomethacin

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Summary

The use of simple alkyl esters as skin permeation enhancers for indomethacin is described. The esters investigated were methyl acetate, ethyl acetate, butyl acetate, methyl propionate, ethyl propionate, and methyl valerate. Other solvents tested as enhancers were water, ethanol, diethyl succinate, ethyl acetoacetate, dimethyl sulfoxide, and Azone. The steady-state flux of indomethacin as measured in vitro through excised rat skin was enhanced about 1500-fold by ethyl acetate, methyl acetate, and methyl propionate relative to that from water. Relative to pure ethanol as permeation enhancing solvent, flux was enhanced over 20-fold using these three solvents. The other alkyl esters were about as effective as ethanol at increasing transdermal flux of indomethacin. Azone (5% in propylene glycol) increased the flux of indomethacin about 1100-fold relative to water and about 14-fold relative to ethanol. Dimethyl sulfoxide (30% in propylene glycol) and ethyl acetate (30% in propylene glycol) were relatively ineffective at increasing the flux of indomethacin. The use of ethyl acetate as a skin permeation enhancer in transdermal drug delivery is discussed.

Introduction

Transdermal drug delivery is generally considered a desirable route of drug administration. However, a major problem encountered with many drugs is the low permeability of human skin, more specifically the stratum corneum, as well as skin's biological variability (Barry, 1983; Schaefer et al., 1982). One way to reduce this problem is to include in the transdermal formulation a chemical or chemicals (permeation enhancers) which reversibly reduces the barrier properties of the skin allowing more drug to penetrate into the viable tissues and the systemic circulation (Barry, 1987a and b; Higuchi et al., 1985; Hadgraft, 1984).

A number of chemicals have been identified as useful permeation enhancers (Barry, 1987a and b; Hadgraft, 1984; Cooper and Berner, 1987; Walters, 1988). Despite the identification of several very effective skin permeation enhancers, such as dimethyl sulfoxide (Stoughton, 1964), Azone ® (1-dodecylhexahydro-2H-azepin-2-one) (Vaidyanathan et al., 1987), dimethylformamide, and dimethylacetamide (Munro and Stoughton, 1965), their usefulness in commercial transdermal products has yet to be demonstrated. Toxicity and skin irritation have generally limited the practical application of these chemicals in transdermal drug delivery systems (Leyden and Grove, 1987; Schmidt, 1988). Thus the search continues for safe, effective, and hopefully generically useful permeation enhancers.

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During our research to develop a transdermal delivery system for levonorgestrel (Friend et al., 1988a and b) we tested ethyl acetate as a potential skin permeation enhancer (Friend et al., 1989a). This common organic solvent was found to be very effective at increasing the transdermal flux of a number of drugs, including indomethacin. Transdermal delivery of indomethacin is desirable in order to reduce the frequency of dosing (normally 4 oral doses per day) and to reduce gastric irritation associated with oral administration. This paper presents the results obtained using a variety of simple alkyl esters along with some other solvents as skin permeation enhancers for indomethacin.

Materials and Methods

Materials

Indomethacin was purchased from Sigma Chemical Co. (St. Louis, MO). Ethyl acetate, methyl acetate, methyl propionate, ethyl propionate, butyl acetate, methyl valerate, diethyl succinate, and ethyl acetoacetate were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. Other solvents were of reagent grade and were used without further purification. Azone was a gift of Nelson Research (Irvine, CA). The rats (male, Wistar strain, 8 weeks old) were obtained from Simonsen Labs, Gilroy, CA.

Permeability studies with rat skins

A system employing 9 glass Franz diffusion cells was used for the permeability experiments with rat skins. The Franz cells were modified with inlet and outlet receiver phase ports to allow continuous flow through the cells.

The rats (180–220 g) were sacrificed in a CO_2 chamber, and an approximately 6 cm^2 area of full-thickness skin was excised from the shaved (electric clippers) abdominal site. After removal of the subcutaneous fat, the skins were washed with physiological saline and used in the permeability experiment within 1 h. The skin was mounted and clamped between the cell body and the cell cap with the furry side facing upward (donor side). The surface area exposed to the donor phase was 5.07 cm^2. The donor phase (ca. 5 ml) was prepared by suspending excess solid indomethacin in the appropriate solvent. The donor phase suspension was applied directly on the skin through the cell cap, which was then sealed with a glass stopper. The receptor phase, in contact with the underside of the skin, was isotonic saline buffered with 0.02 M sodium phosphate, pH 7.4 at 37 °C with 0.1% sodium azide added to prevent bacterial growth. The cells were maintained at 37 °C by thermostatically controlled water which was circulated through a jacket surrounding the cell body. The donor phase temperature was measured at 32 °C.

Receiver phase solution was pumped through the diffusion cells by means of a Manostat Cassette Pump drive unit. A fraction collector was used to collect the cell effluent. The flow rate was set so that the drug concentration in the receptor phase remained below about 10% of saturation; a typical flow rate was 10 ml/h. Uniform mixing of the drug in the receiver phase was achieved by a small magnetic stirring bar driven by an external 600 rpm motor. The donor suspensions were changed daily to reduce the possibility of dilution with water, which can enter the donor phase by reverse flux from the receptor phase. Fractions were collected every 2 h in test tubes. Flux was calculated by measuring the total amount of indomethacin collected in the 2 h period, then dividing by 2 to obtain an hourly rate.

Chromatographic analysis

Indomethacin concentration in the receptor phase was measured using high-pressure liquid chromatography (HPLC). No sample pretreatment was required. The HPLC analyses were performed on a Waters 840 system consisting of two Model 510 pumps, a Model 481 UV detector, a Model 710B WISP (sample processor), and a Digital Computer Model 350 microprocessor/programmer. The column used was a 4.6 mm × 25 cm, 10 μm, Whatman ODS-3 Partisil C-18. A mobile phase of acetonitrile/0.01 M sodium phosphate (pH 7.4) (3 : 7, v/v) was used at a flow rate of 1.8 ml/min with absorbance monitoring at 243 nm. The retention time of indomethacin under these conditions was 4.6 min.

The alkyl esters were analyzed by HPLC using a 7.8 mm × 15 cm Waters Fast Fruit Juice Column, and a Waters Model R-400 Differential Refractometer. The mobile phase consisted of 0.05% (v/v) phosphoric acid in water. A flow rate of 1.8 ml/min was used, and the retention times of the esters were as follows: ethyl acetate, 5.6 min; ethyl acetoacetate, 5.6 min; methyl acetate, 5.7 min; methyl propionate, 6.2 min; ethyl propionate, 7.9 min; diethyl succinate, 10.6 min; butyl acetate, 14.1 min; methyl valerate, 15.1 min.

Partition coefficients and solubility measurements

The octanol/water partition coefficients of the alkyl esters were determined at 24° C. The aqueous phase was buffered to pH 7.4 with 0.02 M sodium phosphate. Equal portions (5 ml) of 1-octanol and buffer were used. The alkyl ester was added to the octanol at 60 μl/ml and the two phases mixed by inversion in a screw-top test tube for 5 min. After centrifuging (500 *g*, 5 min) to ensure separation of the phases, the aqueous phase was analyzed for ester content using HPLC. The concentration of ester in the octanol phase was determined by difference. The partition coefficients of each ester were measured in triplicate.

The solubility of indomethacin in the solvents tested as enhancers was determined as follows. An excess of indomethacin was suspended in the appropriate solvent at 32° C. These suspensions were stirred for 48 h at which time the samples were centrifuged (5000 *g* 10 min, 32° C). The concentration of indomethacin in the supernatant was measured using HPLC as described above and is expressed as mg/ml of solvent.

Results

Eight alkyl esters were evaluated as permeation enhancers for indomethacin: ethyl acetate (EtAc), methyl acetate (MeAc), methyl propionate (MePr), ethyl propionate (EtPr), methyl valerate (MeVal), butyl acetate (BuAc), diethyl succinate (DESuc), and ethyl acetoacetate (EtAAc). Also tested as permeation enhancers were water (0.05 M sodium phosphate, pH 3.0), ethanol, EtAc in ethanol (30% v/v), EtAc in propylene glycol (PG) (30%, v/v),

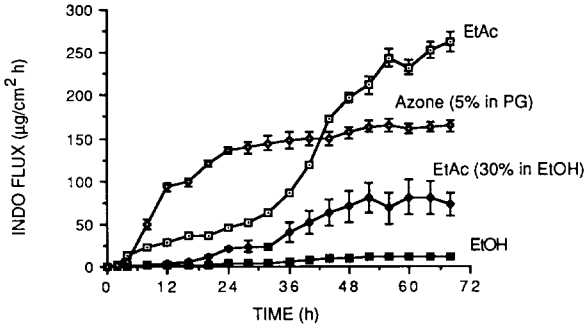

Fig. 1. Flux of indomethacin (INDO) through rat skin in vitro using EtAc ($n = 3$), Azone (5% in PG) ($n = 3$), EtAc (30% in EtOH) ($n = 3$), and EtOH ($n = 3$) as vehicles. Error bars are ± S.E.M.

azone in PG (5%, v/v), and dimethyl sulfoxide (DMSO) in PG (30%, v/v). The aqueous vehicle was adjusted to pH 3 to suppress ionization of indomethacin. Each solvent, or cosolvent system, was saturated with excess solid drug to ensure an equal and constant driving force in all the vehicles tested. Thus differences in flux can be attributed to the effect of solvent on the barrier properties of skin. This assumes that the stratum corneum is the rate limiting element for all solvents tested and that dissolution control is not occurring under the conditions employed.

Indomethacin flux from each donor phase vehicle tested is shown in Figs. 1–4. For most of the vehicles it appears that steady-state flux was reached within 24–48 h. The flux of indomethacin

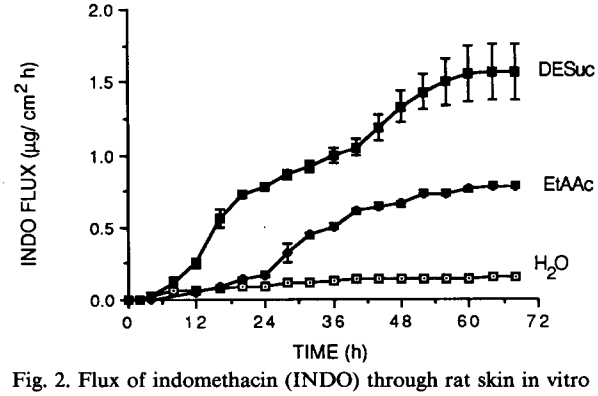

Fig. 2. Flux of indomethacin (INDO) through rat skin in vitro using DESuc ($n = 3$), EtAAc ($n = 3$), and H_2O ($n = 3$) as vehicles. Error bars are ± S.E.M.

TABLE 1

Vehicles, steady-state fluxes, solubilities, partition coefficients of enhancers, boiling points, and enhancement factors

Vehicle	Indomethacin Flux [a] ($\mu g/cm^2 \cdot h$)	Indomethacin solubility (mg/ml) [b]	Log K [c]	Boiling pt. (°C)	Enhancement factors [d]
Water (pH 3)	0.15	0.001 [e]		100	1
EtOH	12	28	−0.35	78	80
EtAc (30% in EtOH)	80	62			530
EtAc	230	42	0.75	77	1 500
MeAc	240	104	0.62	58	1 600
MePr	260	47	0.84	79	1 700
EtPr	100	28	1.3	99	650
MeVal	19	27	1.8	128	125
BuAc	9	27	1.7	126	60
DESuc	1.6	30	1.2	218	5
EtAAc	0.8	36	0.25	181	0.5
Azone (5% in PG)	165	11			1 100
EtAc (30% in PG)	5.5	55			35
DMSO (30% in PG)	3	110			20

[a] Measured at apparent steady-state, donor phase: 32 °C, receptor phase 37 °C.
[b] Measured at 32 °C.
[c] Octanol/water, measured at 24 °C.
[d] Enhancement factor relative to flux from water, pH 3.
[e] from Inagi et al., 1981.

from 5% Azone in PG took about 24 h to reach steady-state (see Fig. 1).

The steady-state flux of indomethacin for each donor vehicle tested is summarized in Table 1. Also included in Table 1 are the permeation enhancement factors relative to water, the octanol/water partition coefficient and boiling point of each solvent, and the solubility of indomethacin in each of the solvents tested. There was no direct relationship between drug solubility in the enhancer solvent and steady-state flux. Thus, the effectiveness of each donor solvent appears to be a result of specific enhancer/skin interactions.

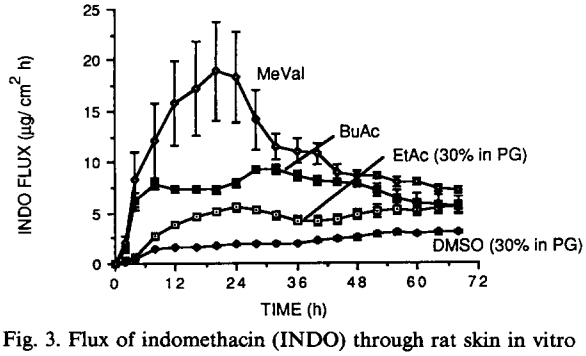

Fig. 3. Flux of indomethacin (INDO) through rat skin in vitro using MeVal ($n = 3$), BuAc ($n = 3$), EtAc (30% in PG) ($n = 3$), and DMSO (30% in PG) ($n = 3$) as vehicles. Error bars are ± S.E.M.

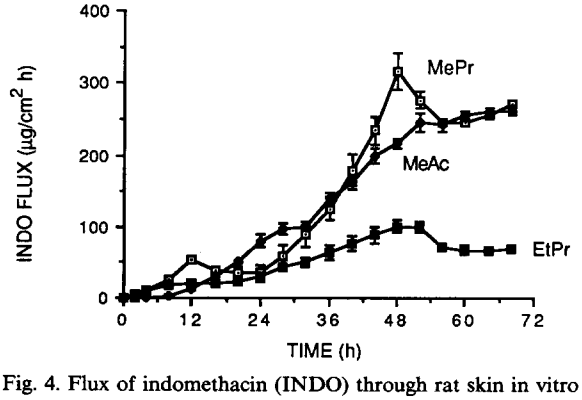

Fig. 4. Flux of indomethacin (INDO) through rat skin in vitro using MePr ($n = 3$), MeAc ($n = 3$), and EtPr ($n = 3$) as vehicles. Error bars are ± S.E.M.

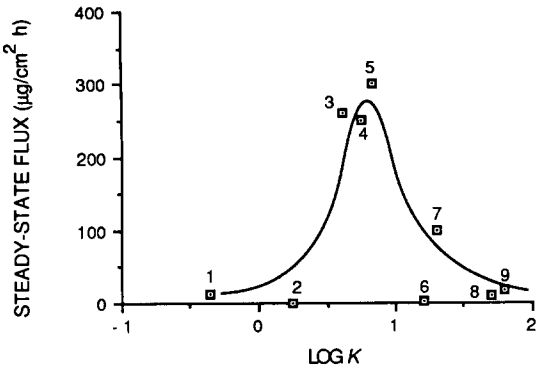

Fig. 5. Relationship between steady-state flux and log K for the vehicles tested. 1: EtOH; 2:EtAAc; 3: MePr; 4: EtAc; 5: MePr; 6: DESuc; 7: EtPr; 8: BuAc; and 9: MeVal.

EtAc, MeAc, and MePr were the most effective permeation enhancers for indomethacin. Relative to water, EtAc increased the flux of indomethacin at steady-state about 1500-fold. Relative to ethanol, the flux enhancement was over 20 times greater. Furthermore, a dilution of 30% EtAc/70% EtOH (v/v) enhanced the flux of indomethacin 7-fold over ethanol alone.

While there were no major discernible trends observed in the data collected, several interesting relationships were noted. Generally, as the alkyl chain length increased, the enhancement effect decreased. Since the alkyl chain length affects the polarity of the esters, the partition coefficient of each ester was measured (see Table 1). When flux of indomethacin through rat skin from the esters tested is plotted against the octanol/water partition coefficient of those esters, an interesting relationship is observed. This is shown in Fig. 5. Maximum enhancement is reached for the esters having a log K of 0.6–0.8. A similar effect was observed for levonorgestrel, another lipophilic drug (Friend et al., 1989a). Two of the solvents tested, DESuc and EtAAc, were poor enhancers compared to the other esters tested despite the fact that these two solvents have partition coefficients close to that of EtAc (see Table 1). It was also noted that, in general, as the boiling point of the ester increased, the flux enhancement of indomethacin decreased.

Azone is an effective permeation enhancer for many drugs (Vaidyanathan et al., 1987) including indomethacin (Sugibayashi et al., 1988; Ogiso et al., 1986); PG has been used as a cosolvent with Azone when tested as a permeation enhancer for indomethacin (Ogiso et al., 1986). Therefore, a mixture of 5% azone in PG was tested for its ability to increase the transdermal flux of indomethacin. As shown in Table 1, Azone was not quite as effective an enhancer as neat EtAc. While indomethacin flux from 5% Azone/95% PG (165 $\mu g/cm^2 \cdot h$) was 14 times higher than the flux from ethanol, it was somewhat lower than the flux from neat EtAc (230 $\mu g/cm^2 \cdot h$) at steady-state; however, steady-state was reached sooner with Azone.

EtAc (30% in PG) and DMSO (30% in PG) were also tested as vehicles. The resulting flux values were quite low (see Table 1). The flux of indomethacin from 30% EtAc/70% PG was much lower than from 30% EtAc/70% EtOH, indicating that EtOH is a good cosolvent for EtAc. We have observed that the combination of EtAc and EtOH is effective with other drugs, such as estradiol and levonorgestrel (Friend et al., 1989a). The low drug flux from 30% DMSO in PG is not unexpected, since in general, the vehicle should contain at least 80% or more DMSO for effective flux enhancement (Munro and Stoughton, 1965; Feldmann and Maibach, 1966).

Discussion

Alkyl esters as permeation enhancers for indomethacin

Indomethacin is a good candidate for transdermal drug delivery; however, relatively large doses are required for treatment of chronic inflammatory disorders. It has been suggested that permeation enhancers could possibly increase the percutaneous absorption of indomethacin so that a therapeutically effective dose can be delivered from a patch of reasonable size (Guy and Hadgraft, 1987). Enhancers tested with indomethacin include Azone, DMSO, isopropylmyristate, diethylsebacate (Sugibayashi et al., 1988; Ogiso et al., 1986); long chain alkanols, alkanoic acids, and esters (Chien et al., 1988); and calcium thioglycolate (Ogiso et al., 1986). It is difficult to compare the enhancement effect of the alkyl esters reported

herein and that of other enhancers due to the very different conditions used.

According to calculations by Guy and Hadgraft (1987), a 100-cm^2 patch would need to deliver indomethacin at a rate of 50 $\mu g/(cm^2 \cdot h)$ to be efficacious. The experiments reported herein indicate that this rate of delivery may be possible using EtAc as a permeation enhancer since the flux obtained at steady-state was close to 250 $\mu g/(cm^2 \cdot h)$. Rat skin, which is generally more permeable than human skin (Wester and Maibach, 1985), was used in these experiments. Therefore, flux enhancement through human skin is most likely not as great as was observed with rat skin. Nonetheless, the use of EtAc as a skin permeation enhancer may be useful in development of a transdermal delivery system for indomethacin. The use of EtAc in transdermal drug delivery is discussed below.

Alkyl esters as permeation enhancers in transdermal drug delivery

Permeation enhancers (Barry, 1987a and b; Ritschel, 1969; Lorenzetti, 1978), prodrugs (Higuchi and Yu, 1987; Hadgraft, 1985) and iontophoresis (Banga and Chien, 1988) have all been investigated as means to increase percutaneous absorption of drugs. Currently, permeation enhancers are favored in the development of commercial transdermal drug delivery systems although iontophoresis may offer advantages over the other techniques, particularly in the transdermal delivery of peptides.

It is well established that for most drugs, permeation through the bulk of the stratum corneum provides the rate-limiting step in percutaneous absorption. The effect of EtAc on the barrier properties of the stratum corneum is not clear. EtAc may be extracting lipids from the stratum corneum leading to reduced diffusional resistance. EtAc has been used to study sebum production and delivery in humans (Millns and Maibach, 1982). These workers found EtAc to be a very good delipidizing agent in vivo. The mechanism of action of EtAc is currently under investigation in this laboratory.

The effect of hydration on the fluxes obtained should also be considered. It is well documented that long-term hydration of hairless mouse skin causes increased permeation of solutes (Bond and Barry, 1988a and b; Behl et al., 1980). The use of water as a donor phase solvent can be used to assess the effect of long-term hydration on the permeation of indomethacin through rat skin. From Fig. 5, it can be seen that the flux of indomethacin from a donor phase of buffered water (pH 3) did not change appreciably over 68 h. This indicates that rat skin is probably not as sensitive to long-term hydration as is hairless mouse skin. The low pH of the aqueous donor phase may have had a small effect on the overall flux of indomethacin. It has been shown that pH 3 leads to slightly higher water diffusion through excised hairless mouse skin relative to pHs in the range 4–10 (Matoltsy et al., 1968).

In using EtAc as a permeation enhancer for transdermal drug delivery systems, a variety of factors must be considered. EtAc is generally recognized as safe (GRAS) by the FDA. It is relatively low in toxicity (LD_{50} for acute oral toxicity is 5–6 g/kg and subcutaneous LD_{50} is 3–5 g/kg). It is hydrolyzed in vivo to ethanol and acetic acid with a $t_{1/2}$ of 5–10 min (Gallaher and Loomis, 1975). EtAc (10% in petrolatum base) has been shown to be non-irritating and non-sensitizing in occlusive patch tests on humans over 48 h (Opdyke, 1974). EtAc affects the horny layer to about the same extent as does EtOH with respect to transepidermal water loss (Malten et al., 1968). We have tested 24-h transdermal devices on rabbits to measure, among other things, the potential irritation of EtAc/EtOH (7 : 3) or pure EtAc under occlusion. The devices induced mild erythema and very mild edema; both changes were reversible (Friend et al., 1989b).

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