ELSEVIER

International Journal of Pharmaceutics 118 (1995) 113–119

international journal of pharmaceutics

Preparation and enhancing effect of 1-(*N,N*-dimethylamino)-2-propanol dodecanoate (DAIPD) on the transepidermal penetration of clonidine, hydrocortisone, and indomethacin through shed snake skin

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Received 20 January 1994; revised 24 October 1994; accepted 27 October 1994

Abstract

1-(*N,N*-Dimethylamino)-2-propanol dodecanoate (DAIPD), a new biodegradable penetration enhancer was prepared by condensing lauroyl chloride with 1-(*N,N*-dimethylamino)-2-propanol. The penetration enhancing effects of DAIPD on the transdermal delivery of clonidine, hydrocortisone, and indomethacin were evaluated using shed snake skin (*Elaphe obsoleta*) as the model membrane. Azone was used as a typical enhancer for comparison. DAIPD showed a 1.2–2-times greater promoting effect than azone for the above-cited drugs. In vitro biodegradability of DAIPD was demonstrated in the presence of porcine esterase. The results indicate that DAIPD increases markedly the transdermal delivery of several types of drug substances.

Keywords: Transepidermal penetration; Shed snake skin; 1-(*N,N* Dimethylamino)-2-propanol dodecanoate; Azone; Enhancer; Clonidine; Hydrocortisone; Indomethacin

1. Introduction

Numerous strategies for delivery of drug substances through the skin have been reported (Kydonieus and Berner, 1987; Pfister and Tsieh, 1990; Ghosh and Banga, 1993a,b). The use of chemical penetration enhancers is a promising approach to enhance the permeation of biologically active substances (Franz et al., 1992). Ideally, these compounds reversibly reduce the barrier effect of the stratum corneum and allow the diffusant to penetrate to the viable tissues and to enter to the systemic circulation (Southwell and Barry, 1983). Several substances such as azone, oleic acid, dimethyl sulfoxide, diethyltoluamide, pyrrolidones, and dodecylmethyl sulfoxide have been evaluated as enhancers. However, many have not been used in transdermal formulations because of their side effects, possible toxicity,

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SSDI 0378-5173(94)00359-9

irritation, lack of reversibility, inadequate understanding of their penetration enhancing mechanism and their low penetration enhancing activities.

In previous studies (Fleeker et al., 1989; Büyüktimkin et al., 1991a, 1992, 1993; Turunen et al., 1993) from this laboratory, new biodegradable and highly effective enhancers were reported. The impetus for developing biodegradable penetration enhancers is based on the fact that they might be less irritating, less toxic, and may possess a limited time of enhancing activity (Wong et al., 1988; Büyüktimkin, et al., 1991b; Hirvonen et al., 1991). On the basis of these considerations and a view of a possible structure/activity relationship, we report the synthesis of and the permeation promoting effect of 1-(*N,N* dimethylamino)-2-propanol dodecanoate (DAIPD) on the transport of clonidine, hydrocortisone and indomethacin as basic, neutral, and acidic drug substances through shed snake skin as a model membrane. The enhancements due to DAIPD are compared to that of azone, as a typical enhancer.

2. Materials and methods

2.1. Chemicals

Lauroyl chloride, triethylamine, and 1-(*N,N*-dimethylamino)-2-propanol were purchased from Aldrich. Azone was supplied by Nelson Research and Development Co. Porcine esterase and clonidine were purchased from Sigma. All of the other chemicals were of reagent grade and obtained through local outlets. Thin-layer chromatographic silica gel plates (60 F 254) and column chromatographic silica gel (130–270 mesh) were obtained from EM Science.

2.2. Apparatus

IR spectra were recorded on a Perkin Elmer 1420 spectrometer. NMR spectra were taken on a General Electric GE 300 instrument. Mass spectra were obtained with a Nermag 10-10 quadropole mass spectrometer. HPLC was performed using a Spectroflow 783 absorbance detector and a Bio-Rad HPLC pump model 1330.

2.3. Synthesis

Lauroyl chloride (43.2 g, 0.2 M) was added incrementally to 20.6 g (0.2 M) of 1-(*N,N*-dimethylamino)-2-propanol in 250 ml $CHCl_3$ in the presence of triethylamine (30 ml), and stirred for 24 h at room temperature. After filtration of the residue, the reaction mixture was washed three times with water (250 ml each washing) and the organic phase was dried over anhydrous magnesium sulfate. The solvent was evaporated in vacuo. The oily residue was dissolved in ethyl acetate and purified by column chromatography using silica gel as supporting material and the same solvent as the eluent. The reaction course was checked by TLC. Ethyl acetate was used as the solvent for TLC analysis. The visualization was accomplished using iodine vapors. The yield was 95%. The TLC R_f values were 0.21 (ethyl acetate) and 0.74 (chloroform/ methanol (1:1)). IR ($CHCl_3$): γ 2920,2840 (C-H), 1730 (C = O), 1040 (C-O-C) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (3H, t, CH_3), 1.21–1.23 (3H, d, $\underline{CH_3}$-CH), 1.26 (broad s, 18H, $(CH_2)_9$), 2.25 (6H,s, $\overline{N}(CH_3)_2$), 2.29 (2H, t, CH_2-N), 2.46–2.52 (2H, m, CH_2-CO), 5.02–5.08 (1H, m, $\underline{CH}$-CH_3) ppm; MS (EI): m/z (RA %) 285 (10), $\overline{158}$ (45), 145(70), 102(28), 58(100); $C_{17}H_{35}NO_2$ (285.47) requires 285.

2.4. R_m values

The lipophilicity expressed as R_m values was determined according to the method of Seydel and Schaper (1979). TLC plates were impregnated with 5% paraffin solution in diethyl ether. A mixture of acetone and phosphate buffer (0.01 M, pH 7.0) (4:1) was used as the developing system. The R_m value was calculated by the use of following equation:

$$R_m = \log[(1/R_f) - 1]$$

2.5. Apparent pK_a values

The apparent pK_a values were determined by titration of 0.25 mmole of enhancer using aque-

ous 0.1 N HCl as titrant in 50 ml acetonitrile/water mixture (1:1). They were calculated according to Albert and Serjeant (1971).

2.6. Shed snake skins

Shed snake skins were prepared as previously reported (Büyüktimkin et al., 1993). They were stored at −20°C. Before the experiment they were allowed to reach room temperature at least 12 h before use. To reduce the variability of results, one whole snake skin was used for each set of experiments.

2.7. Assay of drugs

Indomethacin, clonidine, and hydrocortisone were assayed by HPLC procedures as described earlier (Büyüktimkin et al., 1993).

2.8. Penetration study

Pieces of shed snake skin (approx. 3 × 3 cm) were pretreated with 15 μl of enhancer (divided into three 5-μl portions) 2 h before the experiment. After mounting the skin on top of a Franz receptor cell filled with pH 7.0 (0.1 M) phosphate buffer, the donor cell was clamped on the top of the receptor cell. An aliquot of 0.5 ml of a suspension of hydrocortisone and indomethacin, prepared by suspending 50 and 25 mg of the drugs, respectively, in 25 ml of the same buffer and stirring for 24 h at 32°C, or a solution of clonidine (2%) in the same buffer were added to the donor cell. The receptor phase was stirred with a magnetic stirrer. At appropriate time intervals, samples were withdrawn and analyzed by HPLC. The surface area of the membrane was 1.8 cm^2 and the receptor compartment had a volume of 8–10 ml.

2.9. Esterase hydrolysis

A 0.1 ml aliquot of porcine esterase (253 U/mg protein) was diluted to 100 ml with pH 7.0 phosphate buffer. The enhancer solution was prepared by dissolving approx. 12 mg (approx. 0.045 mmol) of the enhancer in 10 ml acetonitrile. 100 μl of this solution was transferred into a 10 ml volumetric flask. 9.8 ml pH 7.0 buffer and 100 μl diluted esterase solution were added. The mixture was kept in a water bath at 32°C with constant stirring. The disappearance of the enhancer peak was monitored by HPLC. The absorbance wavelength was 204 nm. The solvent system was a mixture of acetonitrile and 0.02 M aqueous sodium hexanesulfonate (7:4) with a flow rate of 0.9 ml/min. The retention time of DAIPD was 3.95 min. The kinetic runs were performed in triplicate.

3. Results and discussion

In previous studies from our laboratory we reported the synthesis and good penetrating enhancing activity of some alkyl *N,N*-dimethylamino alkanoates (Büyüktimkin et al., 1991a, 1992, 1993; Turunen et al., 1993). To evaluate the roles of different moieties of this structure on transdermal delivery, we reversed the OCO functionality to COO structure. Therefore, instead of alkanol derivatives of these enhancers, DAIPD as a representative of alkanoic acid derivatives was synthesized. DAIPD was prepared by reacting 1-(*N,N*-dimethylamino)-2-propanol with lauroyl chloride in the presence of trimethylamine as an acid scavanger. Pure substance was obtained after extensive silica gel column chromatography. The structure of the compound was verified by IR, ^{1}H-NMR and mass spectrometry (Fig. 1).

The penetration profiles of clonidine, hydrocortisone, and indomethacin in pH 7.0 buffer and in the presence of DAIPD and azone at 32°C are shown in Fig. 2 and Tables 1–3. Clonidine as an example of a basic, hydrocortisone as neutral, and indomethacin as acidic drugs were selected as permeants. Shed snake skin was used as the model membrane. The penetration promoting ac-

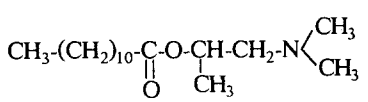

Fig. 1. Structure of DAIPD.

tivity of DAIPD was compared to that of azone as a typical enhancer.

The most remarkable permeation promoting effects were obtained with clonidine and hydrocortisone when compared to azone (the enhancements were approx. 2-fold). Compared to the control, the greatest enhancement was achieved with DAIPD for hydrocortisone. The *n*-octanol/pH 7.0 buffer, partition coefficients of the drugs were reported as log *P* 0.4, 0.8, and 1.2 (Büyüktimkin et al., 1995a) for clonidine, hydrocortisone, and indomethacin, respectively. Indomethacin which is the most lipophilic of the three drugs showed an enhancement approximately equal to that of azone. It was expected that indomethacin as a more lipophilic compound may exhibit a stronger transdermal penetrating effect. However, this order was not followed under the present conditions. These findings suggest that in addition to lipophilicity, other factors such as drug-enhancer and drug-skin interactions also may play a role.

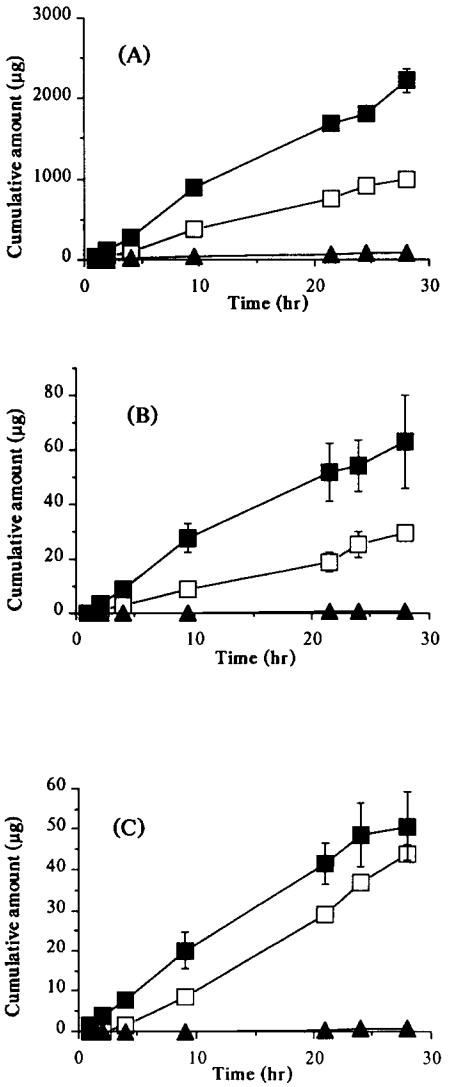

Fig. 2. Penetration profiles of clonidine (A), hydrocortisone (B), and indomethacin (C) in the presence of DAIPD (■), azone (□), and control (▲). Each point and bar show the mean and S.E. of three to five experiments.

It was reported earlier (Büyüktimkin et al., 1993) that DDAIP (dodecyl 2-(*N,N*-dimethylamino)propionate), a new enhancer, increases the permeation of these drugs by a factor of 1.7-, 2.4-, and 4.7-times, respectively, compared to azone. For DAIPD and DDAIP, apparent pK_a values of 8.39 and 6.55 were determined by a potentiometric method. These data show that the enhancers are partly ionized under the experimental conditions. It is possible that complexation with indomethacin may influence the permeation of the drug by forming a derivative of different lipophilicity and ionization. We also observed that DDAIP interacts with indomethacin due to the formation of hydrogen bonding between the carboxylic acid group of the drug and the tertiary amine group of the enhancer. Also, dipole-dipole interactions with these entities may facilitate the penetration enhancing effect (Büyüktimkin, et al., 1995b). Ion-pair formation with the carboxylate anions and cationic amine functionality is assumed by Green et al. (1989) to be responsible for the enhanced transport of β-blocking agents. It was reported (Fleeker et al., 1989) that at pH 7.0 where indomethacin is mainly in the ionized form, the permeability coefficient of the nonionized species is much higher than that of the ionized species in the absence of enhancer. Under the present experimental conditions where the enhancer is mostly in the unionized form but indomethacin is mostly in the ionized form, interaction of both compounds is highly probable. As an indication of the relatively lipid solubility of enhancers, R_m values were compared. The re-

Table 1
Effects of DAIPD and azone on the penetration of clonidine through shed snake skin at 32°C and pH 7.0 ($n = 3$–5)

Compound	Slope [a]	Intercept	r [c]	R.E. [d]	Flux [e]	Permeability [f]
DAIPD	77.92 (4.98) [b]	2.202	0.992	27	42.86	2.14×10^{-2}
Azone	37.56 (0.32) [b]	−28.08	0.996	13	20.84	1.04×10^{-2}
Control	2.90 (0.46) [b]	2.29	0.990	1	1.606	0.8×10^{-4}

[a] Slope of the regression line.
[b] Standard deviation.
[c] Correlation coefficient of the regression line.
[d] Relative enhancement.
[e] μg/h per cm^2.
[f] cm/h.

sults show that they both have similar lipophilicities; 0.194 and 0.212 for DDAIP and DAIPD respectively. All of these data together with pK_a values give some rationale for the relatively low enhancement of indomethacin with DAIPD when compared to DDAIP. Relatively greater enhancements of clonidine and hydrocortisone may be related to the greater solubilization effect of DAIPD or its interaction with different entities of the stratum corneum. In some cases DAIPD may have an advantage over DDAIP in that the synthesis and purification are easier and more rapid.

The existence of esterase activity has been reported in shed snake skin (Nghiem and Higuchi, 1988). To confirm the biodegradability of DAIPD,

Table 2
Effects of DAIPD and azone on the penetration of hydrocortisone through shed snake skin at 32°C and pH 7.0 ($n = 3$–5)

Compound	Slope [a]	Intercept	r [c]	R.E. [d]	Flux [e]	Permeability [f]
DAIPD	2.31 (0.35) [b]	−3.24	0.989	88	1.28	3.56×10^{-2}
Azone	1.06 (0.31) [b]	−1.37	0.988	41	0.59	1.64×10^{-2}
Control	0.026 (0.013) [b]	0.037	0.958	1	1.46×10^{-2}	0.4×10^{-4}

[a] Slope of the regression line.
[b] Standard deviation.
[c] Correlation coefficient of the regression line.
[d] Relative enhancement compared to control.
[e] μg/h per cm^2.
[f] cm/h.

Table 3
Effects of DAIPD and azone on the penetration of indomethacin through shed snake skin at 32°C and pH 7.0 ($n = 3$–5)

Compound	Slope [a]	Intercept	r [c]	R.E. [d]	Flux [e]	Permeability [f]
DAIPD	1.89 (0.21) [b]	−0.76	0.992	53	1.05	1.14×10^{-3}
Azone	1.66 (0.17) [b]	−1.94	0.992	46	0.92	1.01×10^{-3}
Control	0.03 (0.003) [b]	−0.02	0.997	1	0.02	0.18×10^{-4}

[a] Slope of the regression line.
[b] Standard deviation.
[c] Correlation coefficient of the regression line.
[d] Relative enhancement compared to control.
[e] μg/h per cm^2.
[f] cm/h.

its fragmentation in the presence of porcine esterase was examined. The plot of the logarithms of the peak heights vs time indicates that the degradation follows pseudo first-order kinetics, with a k_{obs} of 0.0087 min^{-1} and $t_{1/2}$ 79.5 min (Fig. 3). Esterase catalyzed biodegradability of the enhancer is therefore confirmed. Hirvonen et al. (1991) showed that the transdermal delivery of propranolol is no longer enhanced following pretreatment with DDAA (dodecyl *N,N*-dimethylamino acetate), a biodegradable enhancer, after 4 days whereas with azone the enhancement still existed after a week. Therefore, if they exist, the irritating and toxic effects of biodegradable enhancers can be considerably reduced and the activity period will be limited.

The penetration profiles of the penetrants examined here showed a lag time followed by a linear steady-state flux. The lag times were calculated according to well established methods and listed in Table 4.

An examination of the lag times alone does not provide an answer for the relatively lower enhancement of indomethacin. For clonidine enhancement no lag time was found in the presence of DAIPD. With hydrocortisone the lag time is approx. 45 min whereas with indomethacin it is approx. 24 min. Pretreatment with azone also shows higher lag times suggesting that besides lipophilicity, other mechanisms influence the fluxes of these drugs.

Overall, the results demonstrate that DAIPD is a biodegradable enhancer which is generally more effective than azone. It potentially may be useful in the permeation enhancement of several different classes of drugs.

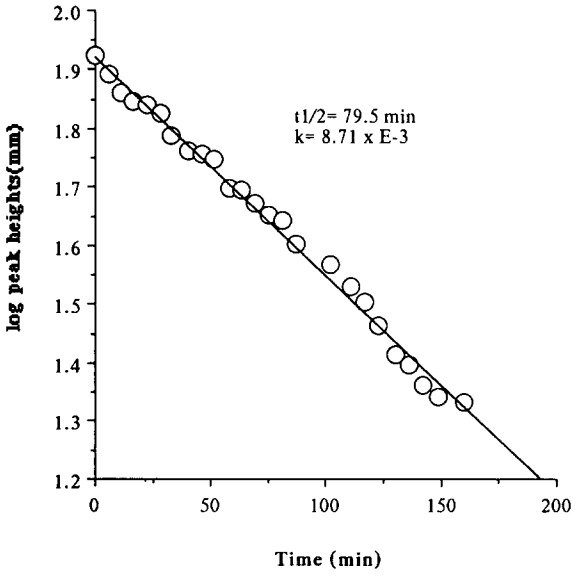

Fig. 3. Biodegradability of DAIPD in the presence of porcine esterase at 32°C and pH 7.0.

Table 4
Lag times of the permeation of clonidine, hydrocortisone and indomethacin in the presence of azone and DAIPD

Drugs	Lag times (min)	
	Azone	DAIPD
Clonidine	45 (3) [a]	0
Hydrocortisone	47 (5)	42 (5)
Indomethacin	69 (7)	24 (7)

[a] Standard deviation.

Acknowledgements

This work was supported by Odontex Inc., Lawrence, KS.

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