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Enhanced delivery of 5-fluorouracil through shed snake skin by two new transdermal penetration enhancers [1]

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Summary

The effectiveness of a new biodegradable penetration enhancer, dodecyl *N,N*-dimethylamino isopropionate (DDAIP) was compared to dodecyl *N,N*-dimethylamino acetate (DDAA), another biodegradable penetration enhancer, and to Azone®, lauryl alcohol (LA), and oleic acid (OA) in vitro using shed snake skin in modified Franz-type diffusion cells. 5-Fluorouracil (5FU), a hydrophilic drug with poor skin permeability, was used as a model permeant. Skin samples were pretreated with pure liquid enhancers. 5FU flux through the control and enhancer treated skin increased linearly with its concentration in the donor compartment. DDAIP and DDAA interacted with the skin rapidly (< 2 h), and the duration of action is at least 24 h. The transdermal flux of 5FU increased substantially (> 40-fold) with the dose of DDAIP up to 10 μl. Thereafter the flux did not increase significantly. With DDAA no plateau effect was observed with the applied volumes up to 50 μl. Skin pretreatment with 5 μl of DDAIP, DDAA, Azone®, LA, and OA increased the permeability of 5FU 69-, 24-, 23-, 5-, and 2-times, respectively. Substitution of a methyl group for a hydrogen atom in the acetate moiety of DDAA was observed to markedly increase transdermal penetration enhancement.

Introduction

The transdermal route has been recognized as a noteworthy alternative for systemic drug delivery with several advantages over conventional routes (Barry, 1983; Kydonieus, 1987; Brown and Langer, 1988). Most drugs do not have a suitable combination of properties needed to penetrate the skin significantly. Therefore, research on chemical penetration enhancing agents has increased. Their clinical use, however, may be limited by their possible skin irritation, accumulation in the skin and subsequent long-term effects, and their potential systemic toxicity (Barry, 1985; Wilhelm et al., 1991). Biodegradable penetration en-

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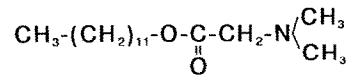
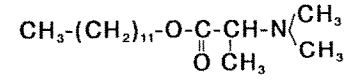
hancers provide an attractive alternative in terms of safety. Dodecyl *N,N*-dimethylamino acetate (DDAA) developed as a biodegradable transdermal permeation enhancer has been shown to be effective in increasing the skin permeation of several drugs using snake, human, and rabbit skin (Fleeker et al., 1989; Wong et al., 1989; Hirvonen et al., 1991a). DDAA is at least as potent penetration enhancer as Azone®, but it is less irritating and shorter acting (Hirvonen et al., 1991b).

The aim of this study was to determine the enhancement effects of dodecyl *N,N*-dimethylamino isopropionate (DDAIP) (Büyüktimkin et al., 1993b), a new derivative of DDAA, containing an additional methyl group, compared to that of DDAA, oleic acid, lauryl alcohol, and Azone®. 5-Fluorouracil (5FU) was chosen as the model drug in the present study because of its poor cutaneous permeability, which is a problem in its clinical use (Ebner, 1970; Klostermann, 1970; Epstein, 1985).



Dodecyl N,N -dimethylaminoacetate



Dodecyl N,N -dimethylamino isopropionate

Fig. 1. The structures of DDAA and DDAIP.

Materials and Methods

5-Fluorouracil, lauryl alcohol and oleic acid were obtained from Sigma Inc. (St. Louis, MO). Azone® was a gift of Nelson Research (Irvine, CA). Dodecyl *N,N*-dimethylamino acetate (DDAA) (Wong et al., 1989) and dodecyl *N,N*-dimethylamino isopropionate (DDAIP) (Büyüktimkin et al., 1993b) were prepared as described previously. The purity of DDAA and DDAIP was more than 99%. The structures of DDAA and DDAIP are presented in Fig. 1. All the other chemicals were reagent grade and were used as received.

Skin preparation

Shed skin of the black rat snake (*Elaphe obsoleta obsoleta*) was used as the model membrane. The skin was hydrated by placing it in water at ambient temperature in order to make it softer and easier to handle. The dorsal portion of the skin was cut into appropriate sizes and stored at −20°C prior to use.

For the pretreatment study, pure liquid enhancer was applied on the dry snake skin and spread out to cover the whole diffusional area prior to the beginning of an experiment. Different amounts (μl) of enhancer and varying pretreatment times (h) were used to determine the optimum effect. Any remaining enhancer was blotted after the pretreatment and the skin was mounted in the diffusion cell. In the control group, the pieces of the skin were rehydrated at 40°C for 30 min, and used as such.

In each four sets of experiments (enhancer comparison, effects of enhancer volume, drug concentration, and pretreatment time), the same snake skin or skins shed during the same week were used, if possible. The absolute permeabilities between different sets of experiments may vary, but within each set the values are comparable.

Evaluation of DDAA and DDAIP

The 5FU permeation study was carried out using Franz-type vertical diffusion cells with an effective diffusional area of 1.8 cm^2. The drug was dissolved in pH 7.2 isotonic phosphate buffer (0.005 M) and 2.0 ml of the solution was applied to the donor cells. Donor concentrations were varied to test the effect of drug concentration on the permeation flux. Since 5FU transport rate through skin has been reported to be pH dependent (Touitou and Abed, 1985), the pH in the donor compartment was adjusted to 7.2 with 0.1 N NaOH. The cell assembly was then placed in a

waterbath maintained at 32°C. The cells had an 8.5 ml receptor compartment that was filled with the pH 7.2 buffer. The receptor fluid was stirred magnetically. Samples of 0.50 ml were withdrawn from the receptor solution at appropriate time intervals over 24 h and assayed for 5FU using the HPLC method described below. The volume of fluid withdrawn was replaced with fresh buffer, and the data were corrected for the drug mass removed.

For the study of the effect of drug concentration, the concentrations of 5FU in the donor cells were 1.0, 2.0, 5.0, and 10.0 mg/ml. The solubility of 5FU in the pH 7.2 buffer was determined to be 15.1 mg/ml at 32°C. Pieces of shed snake skin were pretreated with 5 μl of enhancer for 12 h and used in the penetration experiment as described earlier.

When studying the effect of the amount of enhancer applied on the skins for the pretreatment, pieces of shed snake skin were pretreated with 5, 10, 20 and 50 μl of enhancer for 12 h before use in the penetration experiment. 5-FU concentration in the donor cells was 5.0 mg/ml.

To examine the effect of pretreatment time, pieces of shed snake skin were pretreated with 5 μl of the enhancer for 2, 6, 12, and 24 h. 5-FU concentration in the donor cells was 5.0 mg/ml.

Comparative study of enhancer effectiveness

The enhancers used for pretreatment of snake skin were Azone®, oleic acid, lauryl alcohol, DDAA, and DDAIP. Based on the data obtained from studies described above, experimental conditions were chosen. 5 μl of enhancer was used to pretreat the skin, which was an adequate amount to produce a significant increase in drug flux. The enhancers were left on the skins for 12 h. The drug concentration in the donor cells was 5.0 mg/ml. The permeation study was otherwise conducted as described earlier.

Determination of R_m values

The R_m values were determined according to the reverse-phase TLC method of Seydel and Schaper (1979) using TLC plates impregnated with 5% paraffin solution in diethyl ether. Acetone and acetone/pH 7.0 buffer (4:1) were used as solvents.

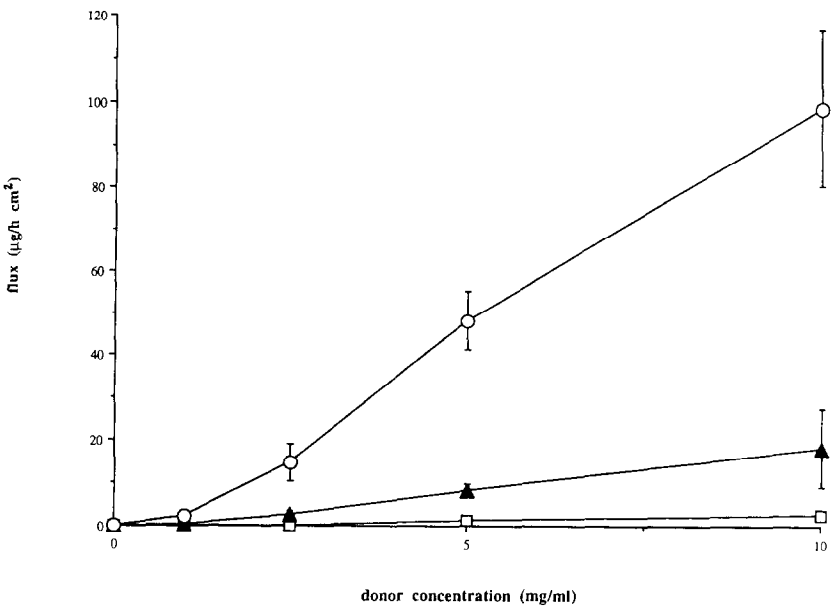


Fig. 2. The effect of donor concentration (mg/ml) on 5FU flux (μg/h per cm^2) through shed snake skin from aqueous solution. Mean ± SD, n = 3–12. (□ ——— □) Untreated, (▲ ——— ▲) DDAA pretreated (5 μl for 12 h), and (○ ——— ○) DDAIP pretreated (5 μl for 12 h).

HPLC assay

A Perkin-Elmer HPLC system with a reversed phase μBondapak-C18 column (10 μm, 3.9 mm × 30 cm, Waters Associates, Milford, MA, U.S.A.) was used to determine the 5FU concentration in the samples at room temperature, with 0.05 M KH_2PO_4-H_3PO_4 buffer (pH 3.0) as the mobile phase. The flow rate was 1.0 ml/min and the UV detection wavelength was 266 nm. Standard solutions were chromatographed and calibration curves were constructed based on peak area measurements. The peak area was linearly related to concentration for samples containing 2–1200 ng of 5-fluorouracil injected onto the column. The correlation coefficient for the standard curve was 0.999.

Data analysis

The data obtained were plotted as the cumulative amount of drug appearing in the receptor compartment as a function of time. The cumulative amount transported was usually linearly related to time. The steady-state flux per unit area, J_{ss} (μg/h per cm^2) may be calculated from the slope of the plot divided by the effective diffusion area and it is directly related to the concentration gradient between the donor and receiver compartments (ΔC) (Scheuplein and Blank, 1971; Michaels et al., 1975) according to Eqn. 1.

$$J_{ss} = k_p \cdot \Delta C \quad (1)$$

This relationship was found to be appropriate for our solution system, since a constantly increased flux with increasing donor concentration was observed (Fig. 2).Thus k_p, the permeability constant (cm/h) can be calculated by dividing the drug flux by the initial drug concentration in the donor phase. Since overall drug permeation across the skin was low, sink conditions were assumed to be maintained throughout the experiment. The significance of the differences between groups was tested using Friedman two-way analysis of variance and Kruskall-Wallis one-way analysis of variance, and thereafter the comparison of each two groups was made by Mann-Whitney's U-test.

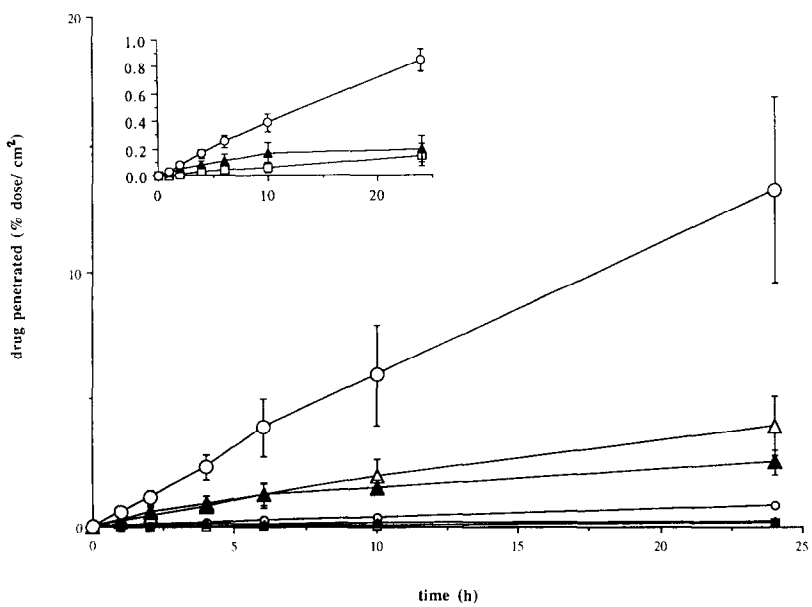


Fig. 3. The permeation of 5FU (% dose/cm^2) from 5.0 mg/ml aqueous solution through untreated (□ ——— □) and enhancer pretreated shed snake skin. Pretreatment with 5 μl of enhancer for 12 h: (▲ ——— ▲) oleic acid, (○ ——— ○) lauryl alcohol, (△ ——— △) Azone®, (▲ ——— ▲) DDAA, (○ ——— ○) DDAIP. The inset illustrates the permeation of 5FU through untreated (□ ——— □), oleic acid treated (▲ ——— ▲), and lauryl alcohol treated (○ ——— ○) snake skin more clearly. Mean ± SD, $n = 4–7$. * $p < 0.05$. Mann-Whitney's U-test compared to Azone® pretreated skin.

Results and Discussion

The time course of 5FU permeation across untreated and enhancer pretreated snake skin is shown in Fig. 3. Drug penetration through untreated skin was very low. 5FU permeability values obtained were consistent with the ones reported earlier (Hirvonen et al., 1991a). The cumulative amount found in the receptor compartment usually displayed a linear relationship with time, and J_{ss} was taken as the slope of the straight line. For DDAA and oleic acid pretreated skins, however, the higher initial rate of transport was followed by a steady-state phase (approx. 4 and 8 h, respectively), after which a 'saturation' phase was observed. In these cases, J_{ss} was taken as the slope of the linear steady-state phase plot. A significant increase in the permeability values (k_p) was observed after pretreatment with each of the enhancers ($p < 0.01$, except for oleic acid $p < 0.05$) (Table 1). The permeability enhancement of Azone® and DDAA was observed to be equal, but DDAIP increased the skin permeability for 5FU significantly more than DDAA or Azone® ($p < 0.05$).

5FU flux was found to be greater for DDAIP than for DDAA ($p < 0.05$) in each case irrespective of the experimental arrangement (Figs 2, 4 and 5). For DDAA, the drug flux increased constantly as more enhancer was applied on the skin

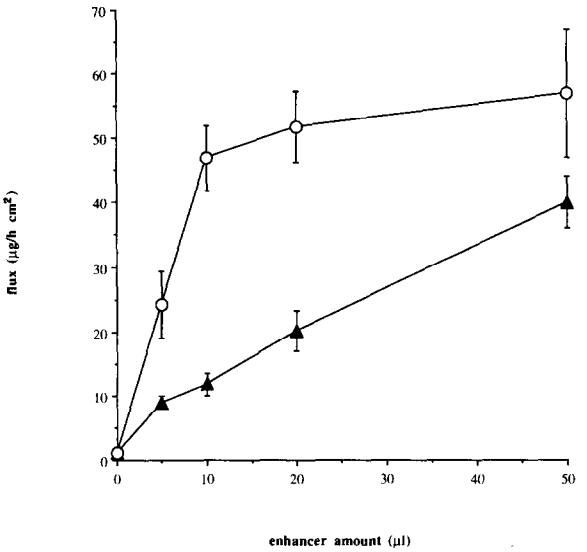


Fig. 4. The effect of the amount of enhancer used for the pretreatment (μl) on 5FU flux (μg/h per cm^2) through shed snake skin from 5.0 mg/ml aqueous solutions. Mean ± SD, $n = 4$–5. (▲ ——— ▲) DDAA, (○ ——— ○) DDAIP.

for pretreatment (Fig. 4). For DDAIP, 5FU flux increased initially more steeply, but amounts of 10 μl or more did not markedly increase the flux further. The difference between DDAIP and DDAA was greatest, 4.0-fold, at 10 μl. Thereafter the difference decreased, being smallest at 50 μl. Since the equilibration time of the enhancer on the skin surface was 12 h, differences

TABLE 1

5FU permeation through untreated and enhancer pretreated (5 μl for 12 h) shed snake skin from a 5.0 mg/ml aqueous solution at 32°C

Treatment of the snake skin	Total drug penetrated (% dose/cm^2) in 24 h	J_{ss} (μg/h per cm^2) [d]	k_p ($\times 10^3$) (cm/h) [e]	Enhancement factor [f]
Untreated	0.2 (0.1) [c]	0.9 (0.6)	0.2 (0.1)	1
Oleic acid	0.2 (0.1)	1.9 (1.1)	0.4 (0.2)	2
Lauryl alcohol	0.9 (0.1)	4.6 (0.4)	0.9 (0.1)	5
Azone®	4.0 (1.1)	21.0 (6.3)	4.2 (1.3)	23
DDAA [a]	2.5 (0.5)	21.7 (12.3)	4.3 (2.5)	24
DDAIP [b]	13.3 (3.7)	62.6 (10.5)	12.5 (2.1)	69

[a] Dodecyl *N,N*-dimethylamino acetate.
[b] Dodecyl *N,N*-dimethylamino isopropionate.
[c] Numbers in parentheses are SD with $n = 4$–7.
[d] Steady-state flux per unit area (mean ± SD).
[e] Permeability coefficient (mean ± SD).
[f] Permeability with enhancer divided by permeability without enhancer.

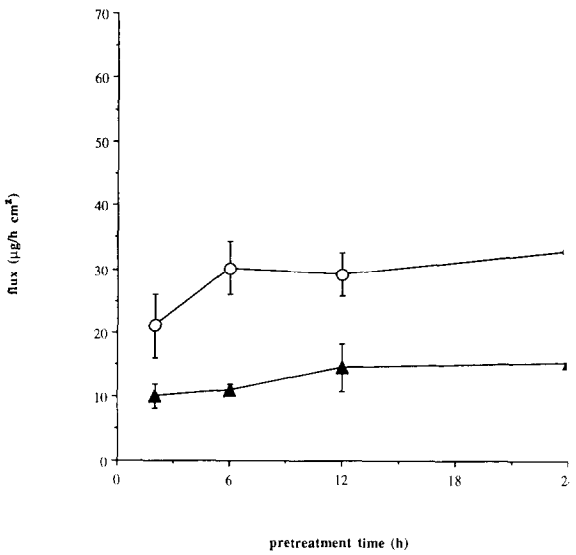


Fig. 5. The effect of pretreatment time (h) on 5FU flux (μg/h per cm^2) through shed snake skin from 5.0 mg/ml aqueous solutions. Mean ± SD, n = 3–5. (▲——▲) DDAA, (○——○) DDAIP.

of the enhancer-skin contact and immersion of the interphase are assumed to be negligible. This is indicated also by Fig. 5, which shows a rapid onset of enhancer action (< 2 h) and time independence between 2 and 24 h of pretreatment. Even the smallest volume, 5 μl, was easily spread on the diffusional area of the skin sample. These data show the importance of the enhancer amount in skin permeability studies. For example, at volumes of 5, 10, 20, and 50 μl the relative differences between DDAIP and DDAA groups were 2.7-, 4.0-, 2.6-, and 1.4-fold, respectively. In order to draw relevant conclusions in transdermal studies, the dose of the penetration enhancers should be addressed. In our study (Fig. 3 and Table 1), 5 μl was used in the comparison of the enhancers. With larger doses oleic acid, lauryl alcohol, and Azone® may have higher enhancement effect, but the difference at 5 μl is extensive, and changes in the order of enhancer activity are not expected at higher doses. For clinical use enhancers should be active at small doses.

The contribution of the methyl group to the greater enhancement effect of DDAIP compared to that of DDAA seems to be important. The difference in the effectiveness of DDAIP and DDAA may be due to (1) different solubilities in the skin, (2) a different concentration-activity relationship in the skin lipid domain, (3) different degrees of degradation in the skin, and (4) different elimination from the skin. As an indication of the relative lipid solubility of these two compounds, R_m values were determined by reverse-phase TLC. DDAIP was found to be significantly more lipophilic (R_m = 0.194) than DDAA (R_m = 0.061) (Buyuktimkin et al., 1992a). In analogy to partition coefficients, the compound with a higher lipophilicity would be expected to be more soluble in or partition into the stratum corneum to a greater extent and exert a greater effect on the SC. The fact that DDAIP with higher lipophilicity shows better enhancement for the delivery of 5FU on snake skin compared to that of DDAA, would be supported by this argument. DDAIP and DDAA have been shown to degrade at different rates in aqueous solutions in the presence of porcine esterase, DDAA ($t_{1/2}$ = 6.8 min at pH 7.0) (Büyüktimkin et al., 1993a) degraded faster than DDAIP ($t_{1/2}$ = 18.5 min at pH 7.0) (Büyüktimkin et al., 1993b). The positive inductive effect of the methyl group neighboring the carbonyl moiety in DDAIP may increase the stability of DDAIP compared to DDAA in the presence of porcine esterase. Although esterase activity has been shown in shed snake skin (Nghiem and Higuchi, 1988), the concentration of the enhancer in the skin after the application of pure enhancer may exceed the V_{max} value of the enzyme(s) substantially. Thus the apparent half-life of the enzymatic degradation of DDAIP and DDAA may be very long in the skin, and consequently, this mechanism is probably not an important determinant in the difference of the two enhancers. Of the remaining mechanisms, the relative importance of enhancer activity, and elimination from the skin is difficult to estimate on the basis of our results.

In conclusion, DDAIP is a more effective penetration enhancer than DDAA, Azone®, and other enhancers tested for 5FU in shed snake skin. It is interesting to notice, that there is only a difference of one methyl group in the structures of DDAIP and DDAA, and yet the difference in their performance in promoting 5FU permeation

is dramatic. Possible structure-activity relationships and true biodegradability remain to be elucidated in further studies.

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