International Journal of Pharmaceutics, 60 (1990) 1–9
Elsevier

IJP 02012

Research Papers

Transdermal delivery of 5-fluorouracil and its alkylcarbamoyl derivatives

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(Received 22 June 1989)
(Modified version received 12 October 1989)
(Accepted 16 October 1989)

Key words: 5-Fluorouracil; Lipophilic prodrug; Alkylcarbamoyl derivative; Percutaneous permeation; Chemical conversion; Keratin binding

Summary

The transdermal absorption of 5-fluorouracil and its alkylcarbamoyl derivatives containing various lipophilic pro-moieties, including butylcarbamoyl, hexylcarbamoyl and octylcarbamoyl groups, was investigated in the rat. All compounds showed various degrees of lipophilicity and instability at physiological pH. Transdermal permeation of these drugs was examined by employing an in vitro technique using a diffusion device which was mounted with a full-thickness rat skin as the diffusion membrane. On application of the alkylcarbamoyl derivatives to the rat skin, 5-fluorouracil appeared in the receptor phase. 1-Butylcarbamoyl and 1-hexylcarbamoyl derivatives showed 3–4-fold greater penetration than that of 5-fluorouracil. A lag time was observed before steady-state diffusion of the drug was established which was prolonged with increasing alkyl chain length. The increase in lipophilicity of the derivatives was found to enhance their binding with keratin, a component of the skin. The accumulation of both derivatives and 5-fluorouracil in the skin was noted. Drugs accumulated rapidly in the skin within 2 h after topical application and reached a constant level thereafter. In particular, the 1-butylcarbamoyl derivative, which permeated rapidly through the skin, showed a high level of accumulation in the skin. Co-application of 5-fluorouracil and the 1-octylcarbamoyl derivative resulted in an additive effect of skin absorption of 5-fluorouracil.

Introduction

Recently, percutaneous drug delivery has attracted a great deal of attention not only in the therapeutic area of local chemotherapy but also in systemic chemotherapy (Shaw, 1982; Chien, 1983). However, most drugs cannot penetrate through a dense and hydrophobic skin barrier at a rate sufficiently high to achieve therapeutic efficacy.

Numerous attempts have been reported to improve absorbability by pharmaceutical approaches. We have also been engaged in enhancing drug delivery via topical skin warming and addition of an absorption enhancer (Sasaki et al., 1987a, 1988). Another promising approach is to develop lipophilic and low melting point prodrugs which revert to the active compound in the skin (Yu et al., 1979, 1980; Bodor et al., 1980; Sloan and Bodor, 1982; Møllgaard et al., 1982; Mukai et al., 1985; Johansen et al., 1986).

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In the prodrug approach, the ability to revert from the prodrug to the active agent via either enzymatic or chemical reactions should be considered as an important factor as the lipophilicity for the improvement of drug effectiveness. Complete and immediate reversion after the prodrug has penetrated the skin barrier is desirable from the standpoints of the efficacy of the parent drug and the potential side effects of the prodrug. However, Pannatier et al. (1978) demonstrated that the metabolizing potential of skin was much lower than that of the liver. Furthermore, enzymatic reaction in the skin depends on the species and substrates (Pannatier et al., 1981; Täuber, 1982; Hashida et al., 1985; Johansen et al., 1986). Enhancement of delivery using prodrugs activated rapidly by chemical lability has been shown to be promising by Sloan et al. (1984a, b).

5-Fluorouracil (**I**) has been used clinically for treatment of carcinoma of the breast and gastrointestinal tract. Topical application of **I** has also been proven to be a valuable treatment for various diseases including epithelial neoplasms and psoriasis (Klein et al., 1970; Tsuji and Sugai, 1972). The topical route of administration can circumvent or at least minimize the systemic toxicity of an anticancer agent. Generally, however, hydrophilic compounds such as **I** cannot penetrate the hydrophobic skin barrier efficiently. Therefore, some prodrugs of anticancer agents which show a high affinity for the stratum corneum of skin have been studied with respect to enhancing transdermal delivery (Yu et al., 1979; Møllgaard et al., 1982; Bundgaard et al., 1983; Sloan et al., 1983, 1984a; Mukai et al., 1985). However, none has emerged as a successful candidate for clinical use.

In a previous report, we prepared alkylcarbamoyl derivatives of **I** and investigated their antitumor activity (Sasaki et al., 1987b). The hexylcarbamoyl derivative (**III**) has been used clinically as an oral antitumor agent (Ozaki et al., 1977). A preliminary study indicated that these derivatives regenerated **I** rapidly at physiological pH. However, they were stable at acidic pH or in organic solvent. These characteristics may be advantageous for transdermal application because the drug will remain as a lipophilic prodrug in the stratum corneum (acidic and lipophilic layers) and will revert to **I** rapidly in the viable cell layer (physiological pH). Therefore, in the present study, their transdermal absorption characteristics were compared using in vitro techniques in rats.

Materials and Methods

Materials

Compounds **I**, **III** and the 1-octylcarbamoyl derivative (**IV**) were supplied by Mitsui Pharmaceuticals (Tokyo). 1-Butylcarbamoyl-5-fluorouracil (**II**) was synthesized as reported by Ozaki et al. (1977). Keratin powder (prepared from wool) was purchased commercially (Nakarai Chemicals, Kyoto). All other chemicals were of reagent grade.

Stability measurement in aqueous solution and biological media

Stability experiments were carried out in pH 6.5 phosphate buffer (0.1 M Na_2HPO_4, 0.2 M NaH_2PO_4, 0.4 M NaCl, 3 : 2 : 2, v/v), pH 4.0 acetate buffer (0.05 M acetate, 0.05 M sodium acetate, 10 : 1, v/v) and isopropyl myristate at 32 ± 0.2° C. The study was conducted as follows: At time zero, a stock solution was added to a preheated buffered solution to give a concentration of 1×10^{-4} M. Aliquots of the solution were withdrawn at appropriate intervals for HPLC assay over a period of 8 h. The half-life of degradation in pH 7.4 phosphate buffer (0.1 M Na_2HPO_4, 0.2 M NaH_2PO_4, 0.15 M NaCl, 11 : 2 : 5, v/v) at 37° C was determined from previous data (Sasaki et al., 1987b).

Lipophilicity and solubility

Apparent partition coefficients of compounds were determined in a chloroform or octanol, pH 4.0 acetate buffer solution system (15 : 2, v/v, for derivatives; 2 : 15, v/v, for **I**) after shaking for 24 h at 37° C. The concentrations of derivatives in the aqueous phase were determined after extraction by chloroform or octanol. Before use, the organic solvent and buffer solutions were saturated with the relevant aqueous or organic phase. The initial concentrations of compounds were 2 mM dissolved in organic solvent (or buffer solution for **I**).

The solubilities in pH 4.0 acetate buffer solution or isopropyl myristate were determined by suspending excess compound in the solvent after shaking for 24 h at 37 °C, followed by filtration and analysis.

In vitro transfer through rat skin

The in vitro diffusion cell was similar to that used by Loftsson and Bodor (1981). The diffusion membranes were full-thickness abdominal skins of male Wistar albino rats weighing 250–300 g. Hair was removed from rats using an animal clipper and a shaver 24 h before experiments. The excised skin was mounted in the diffusion cell and the receptor phase was filled with isotonic sodium phosphate buffered saline (pH 7.4, 49 ml) containing kanamycin sulfate (100 ppm). Test formulations were prepared by suspending drugs (100 μmol) in isopropyl myristate (1 ml). A volume of 1 ml of the formulation was gently applied to the skin surface on the donor side. The available diffusion area was 6.8 cm^2. The diffusion cell was placed in a thermostated chamber maintained at 32 °C, the receptor phase being agitated by a magnetic stirrer. At appropriate intervals, samples of receptor fluid were withdrawn. The samples were immediately acidified (pH < 4) with 0.1 N HCl and then extracted with CH_2Cl_2. After centrifugation for 30 min at 1700 × *g*, the organic and aqueous layers were separated. The organic layer was evaporated to dryness. The residue was dissolved in 0.5 ml acetonitrile for HPLC assay in the case of the derivatives. An aliquot of aqueous layer was used for assay of **I**.

Skin accumulation

Accumulation of derivatives was determined in skin tissue as follows. At the end of an absorption period of 0.5, 2 and 8 h, the skin was removed from the device and washed with 0.1 N HCl. The skin tissue was homogenized with 0.1 N HCl and the homogenate made up to a volume of 50 ml. Drug in this acidic sample was determined in the same manner as receptor fluid sample.

Determination of binding of drugs with keratin

The extent of drug binding with keratin was determined. Keratin powder (100, 200 and 500 mg) was suspended in 6 ml drug solution (0.1 mM) dissolved in pH 7.4 phosphate buffer. After passage through a filter paper (Toyo Roshi, Tokyo), the filtrate was subjected to analysis. Binding was calculated by subtracting filtrate concentration from the initial level of drug.

Analytical methods

HPLC assay was used in these studies. The chromatographic apparatus (LC-5A pump, SIL-1A injector, Shimadzu, Kyoto) was fitted with a UV absorbance detector (SPD-2A variable-wavelength detector, Shimadzu). The stationary phase was a Cosmosil $5C_{18}$ packed column (diameter, 4.6 mm; length, 150 mm; Nakarai Chemicals) and peaks were detected at 265 nm (**I**) and 260 nm (**II–IV**). The column was run at room temperature. A mixture of methanol and distilled water (**II**, 60 : 40, **III**, 72 : 28; **IV**, 82 : 18, v/v) was used as the mobile phase for the derivatives at a flow rate of 1.0 ml/min. Acetate (0.1%) was used at a flow rate of 0.6 ml/min as the mobile phase for **I**. The chromatographic mobile phase was filtered by passing through a 0.45 μm pore size membrane filter (Toyo Roshi). The standard solutions were chromatographed and calibration lines were constructed on the basis of peak-area measurements.

Determination of flux and apparent lag time

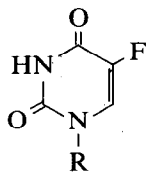
The flux and apparent lag time of penetration of compound **I** after application of compounds **I–IV** were determined from the data on the appearance of **I** via a microcomputer using a nonlinear least-squares method (Yamaoka et al., 1981).

Results and Discussion

The structures and physicochemical properties of compounds **I–IV** are summarized in Table 1. Some of the data have been reported previously (Sasaki et al., 1987b). Compounds **II–IV** showed increased lipophilicity as a result of the introduction of the alkylcarbamoyl group. The partition coefficients of these compounds covered a range of about five orders of magnitude. They exhibited varying degrees of decrease in aqueous solubility. They also possessed lower melting points, suggest-

TABLE 1

Structures and physicochemical properties of 5-fluorouracil and its alkylcarbamoyl derivatives



Compound	R	m.p. (°C)	Log *P*		Solubility (mM)		Half-life (min)[e] under various conditions			
			Chl [a]	Oct [b]	Buffer [c]	IPM [d]	A	B	C	D
I	H	> 270	−2.01	−1.08	> 90	0.08	– [f]	– [f]	– [f]	– [f]
II	$CONH(CH_2)_3CH_3$	130–133	1.66	1.48	4.8	38.1	12	28	– [f]	– [f]
III	$CONH(CH_2)_5CH_3$	112–113	2.78	2.63	0.36	44.9	10	23	– [f]	– [f]
IV	$CONH(CH_2)_7CH_3$	92–94	3.94	3.90	0.03	48.4	9	24	– [f]	– [f]

[a] Logarithmic value of apparent partition coefficient (*P*) between chloroform and pH 4.0 acetate buffer at 37 °C.
[b] Logarithmic value of apparent partition coefficient (*P*) between octanol and pH 4.0 acetate buffer at 37 °C.
[c] Solubility in pH 4.0 acetate buffer at 37 °C.
[d] Solubility in isopropyl myristate at 37 °C.
[e] Half-lives of degradation in pH 7.4 phosphate buffer at 37 °C (A) and pH 6.5 phosphate buffer (B), pH 4.0 acetate buffer (C) and isopropyl myristate (D) at 32 °C.
[f] Degradation was barely detectable for 8 h.

ing an increase in thermodynamic activities of their pure solid state (Higuchi, 1978). The degradation profiles of compounds **II–IV** showed pseudo first-order kinetics in pH 7.4 and 6.5 buffers. The half-lives were calculated from the slopes. Compounds **II–IV** were unstable at physiological pH (pH 6.5 and 7.4) although they were stable at acidic pH (pH 4.0) and in organic solvents such as isopropyl myristate. These results support the conclusion reported by Buur and Bundgaard (1985) in a detailed study of the kinetics of hydrolysis of five 1-carbamoyl-5-fluorouracil derivatives in which they demonstrated that all derivatives hydrolyzed yield compound **I** in a quantitative fashion and that the rates of decomposition in aqueous solution showed a sigmoidal profile for the pH dependence.

The transdermal permeation of **I** and its derivatives was studied by an in vitro technique using a diffusion device mounted with a full-thickness rat skin as the diffusion membrane. In order to compare skin permeation at the drugs' maximum thermodynamic activity, drugs were applied as a suspension in isopropyl myristate. The permeation profiles obtained for compounds **I–IV** in terms of concentration in the receptor phase as a function of time are shown in Fig. 1A–D. Compound **I** attained steady-state diffusion in the skin after a lag time of about 1 h. When alkylcarbamoyl derivatives were applied on rat skin, compound **I** appeared in the receptor phase. These results are in agreement with the finding of the derivatives being unstable at physiological pH. No conversion of derivatives was observed on the donor side. Compounds **II** and **III** resulted in 3–4-fold greater permeation than **I**. Compound **IV** showed comparable permeation to **I**. A lag time was observed before steady-state diffusion of drug was established, which increased in duration for increasing length of alkyl chain of the derivatives.

The profile for the appearance of compound **I** in the receptor phase after topical application of the various prodrugs confirmed the findings on their permeation through the stratum corneum. The conversion of alkylcarbamoyl derivatives to **I** in the viable cell layer beneath the stratum corneum and the subsequent permeation of **I** through viable tissue are believed to be much faster than the permeation of prodrugs through the stratum corneum layer. The solubilized por-

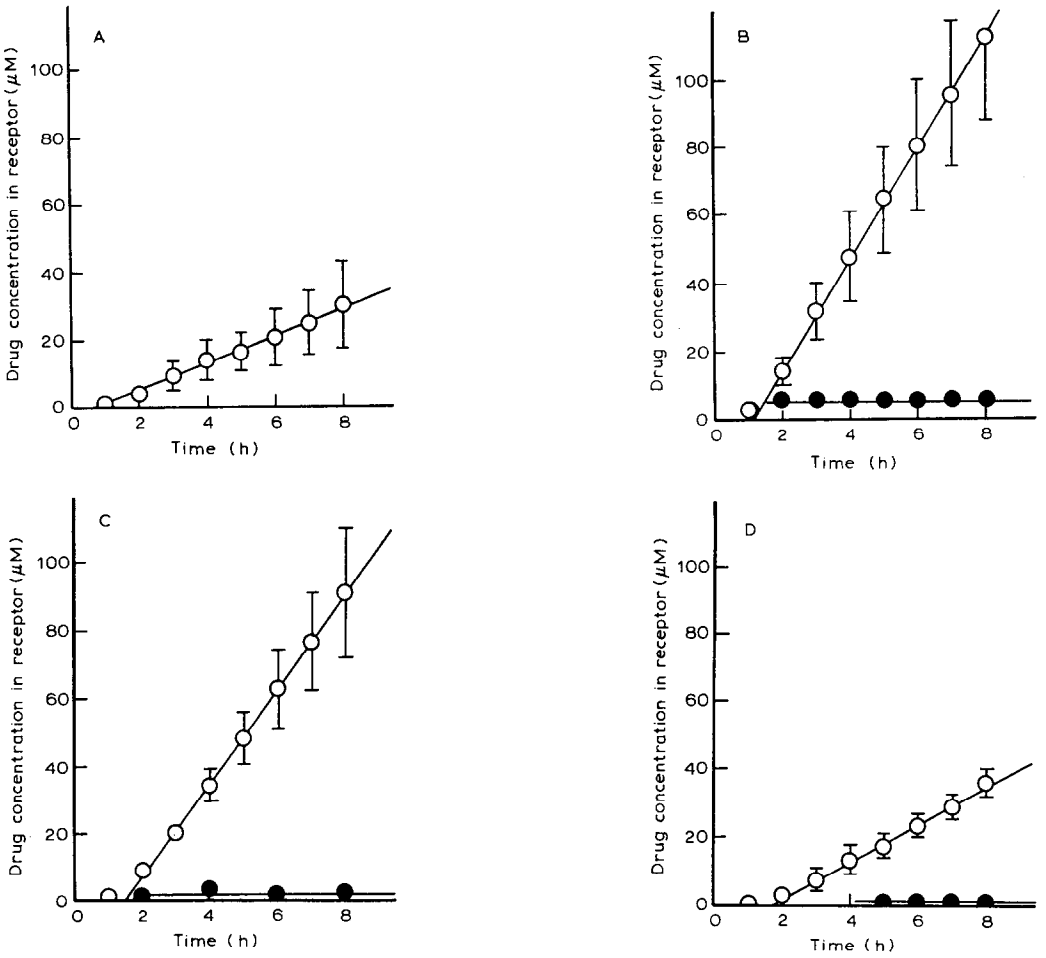


Fig. 1. Percutaneous permeation of 5-fluorouracil and its alkylcarbamoyl derivatives through rat skin in vitro. Appearance in the receptor phase of **I** (○) or derivative (●) after application of **I** (A), **II** (B), **III** (C) and **IV** (D). Vertical bars indicate standard deviations and each point is the mean of at least three experiments.

tion of the dose is more than enough to sustain a constant concentration driving force for diffusion before dissolution sets in as the rate-limiting step. Since the total amount of prodrug permeating through the skin is only about 5% or less of the applied dose at the end of the 8 h experiment, the time course of the appearance of **I** in the receptor phase reflected that of the permeation of derivatives through the stratum corneum.

The flux (dQ/dt) and apparent lag time (LT) of penetration of **I** were determined graphically, yielding the data listed in Table 2. Flynn and Yalkowsky (1972) studied the effect of alkyl chain length on the flux across a synthetic membrane. They found that a plot of the logarithm of steady-state flux from saturated solutions vs chain length gave a parabolic curve. The maximal flux is attained between C-3 and C-4. For chain length increasing beyond C-4, the flux decreased due to diminished aqueous solubility. These results indicate that the mechanism of diffusion passes from regulation by the membrane to being governed by the diffusion layer at about C-4. In the present study, compound **II** (C-4) also showed the highest flux, the flux of prodrug decreasing with greater alkyl chain length of prodrug. On the other hand, they reported that the plot showed a plateau beyond C-4 on normalization of the flux to the

TABLE 2

Flux and apparent lag time of appearance of 5-fluorouracil in receptor phase after application of 5-fluorouracil and its alkylcarbamoyl derivatives on rat skin

Compound	dQ/dt [a] (μmol/cm^2 per h)	LT [a] (h)
I	0.033 ± 0.010	0.82 ± 0.42
II	0.120 ± 0.013	1.17 ± 0.26
III	0.106 ± 0.014	1.62 ± 0.26
IV	0.044 ± 0.004	2.07 ± 0.81

[a] dQ/dt, flux; LT, apparent lag time. Means ± S.E. of at least three experiments.

solubility. However, when the flux of prodrugs was normalized to the solubility in isopropyl myristate in the same manner, the permeability did not show a plateau but decreased with greater alkyl chain length of prodrugs. On normalizing the flux to the solubility in aqueous solution, an increase in permeability occurred for greater alkyl chain length of the prodrugs. Flynn and Yalkowsky (1972) concluded from their analysis that the barrier was a single membrane, sandwiched between two aqueous diffusion layers of identical thickness. The present barrier had three different layers comprising an organic diffusion layer in the donor phase, a membrane diffusion layer of the skin and an aqueous diffusion layer in the receptor phase. The use of isopropyl myristate as a vehicle may be attributable as the source of the deviation from their results. The heterogeneity of the stratum corneum might be also ascribed as the cause of the disagreement.

The apparent lag time (LT) of penetration of prodrugs was prolonged with increasing alkyl chain length of prodrugs as shown in Table 2. Flynn and Yalkowsky (1972) studied the effect of silica filler in the synthetic membrane on the permeation of *p*-aminobenzoate esters. Adsorption prolonged the lag time. They proposed the following correction for their observed lag time: $((h^2/6D) \times (1 + KV))$, where K is the adsorption constant, V is the volume fraction of the adsorbent in the membrane, and D and h are the diffusion constant and membrane thickness, respectively. The considerable lag time for penetration of lipophilic compounds may be explained by their hydrophobic interaction with a lipophilic component of the stratum corneum. Stratum corneum is composed of 80% protein (mainly keratin), 10% lipid and 10% minor component (Flynn, 1979). Therefore, the binding of drug with commercial keratin as a model component of stratum corneum was examined. The results are shown in terms of ratio of drug remaining as a function of keratin concentration in Fig. 2. The increase in lipophilicity of the compounds resulted in the enhancement of keratin binding. We also demonstrated that increased lipophilicity of the compounds enhanced uptake by phospholipid vesicle in a previous study (Sasaki et al., 1987b).

Fig. 3A–D represents the data on accumulation of **I** and its derivatives in whole skin. These experiments were carried out separately from those on transfer. Compound **I** was accumulated rapidly in the skin within 2 h during the early stage after topical application. Thereafter, its concentration maintained fairly constant. This is in good agreement with the observed lag times which ranged from 1.17 to 2.07 h. Washitake et al. (1972) also demonstrated the same trend for the skin accumulation profile of salicylic acid and carbinoxamine. A steady state was reached after the lag time. The rate of drug entry into the skin from the formulation equals that of drug efflux from the skin for the receptor. After application of compounds **II**–

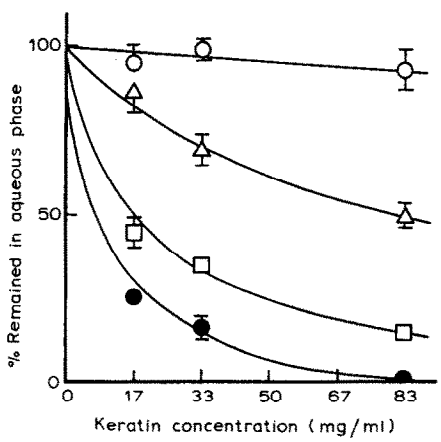


Fig. 2. Binding of 5-fluorouracil and its alkylcarbamoyl derivatives with keratin. (○) **I**, (△) **II**, (□) **III**, (●) **IV**. Vertical bars indicate standard deviations and each point is the mean of at least three experiments.

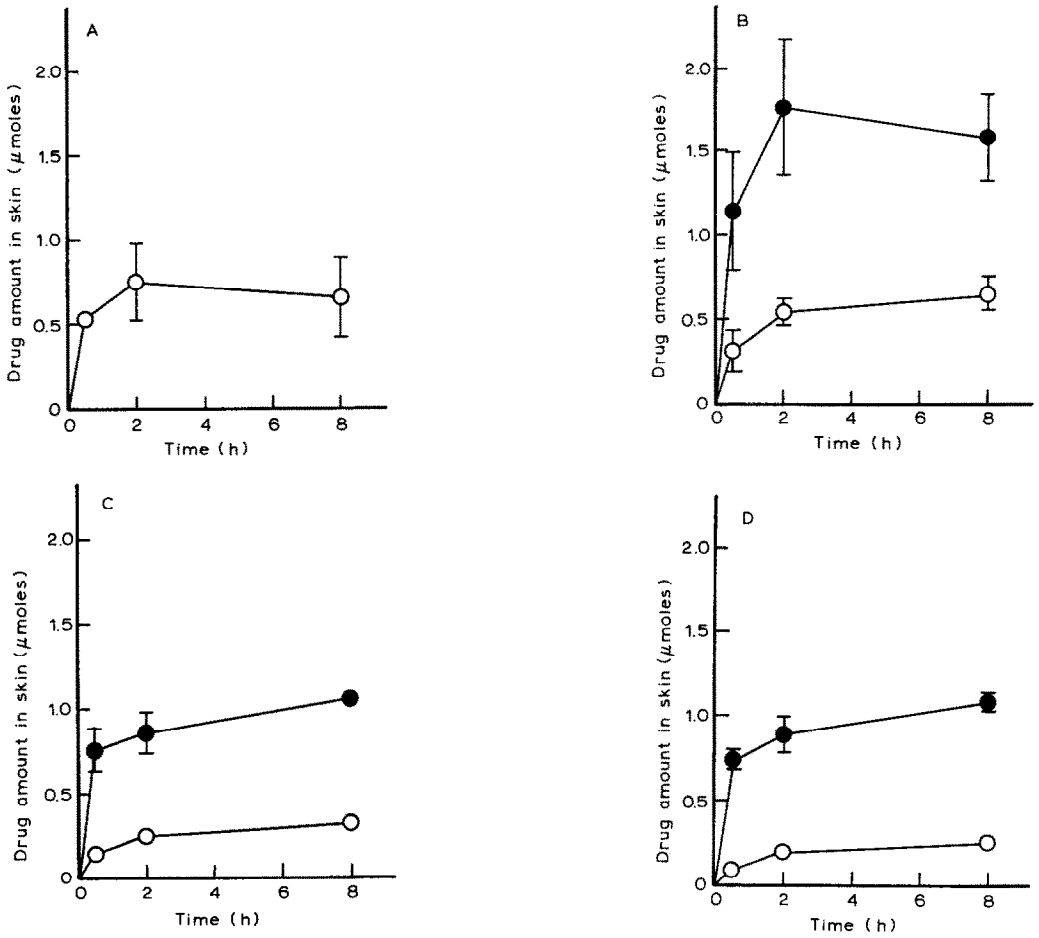


Fig. 3. Skin accumulation of 5-fluorouracil and its alkylcarbamoyl derivatives. Amount in the skin of **I** (○) or derivative (●) after application of **I** (A), **II** (B), **III** (C) and **IV** (D). Vertical bars indicate standard deviations and each point is the mean of at least three experiments.

IV, both compound **I** and derivative were detected in the skin and, in contrast to the results on permeation, greater amounts of the derivatives than that of **I** were found. Most of the derivatives detected may exist in the skin outer layer which has acidic and hydrophobic properties. In this experiment, drug accumulation by the skin reflected not only drug interaction with the stratum corneum but also the drug transfer rate, since the epidermal volume is much larger than that of the stratum corneum. Compound **II**, which showed greatest extent of penetration also demonstrated the highest degree of accumulation in the skin.

Fig. 4 shows the transfer of **I** on co-application with **IV**. Co-application showed an additive effect on the absorption of compound **I**. The co-application of parent drug and prodrug represents a promising approach to the further improvement of absorption.

In conclusion, the physicochemical properties of derivatives are among the most important factors affecting the transdermal absorption of drug. It is suggested from these results that the increase in thermodynamic activity of the derivatives, as expected from their low melting point in comparison to **I**, enhances their uptake into the hydro-

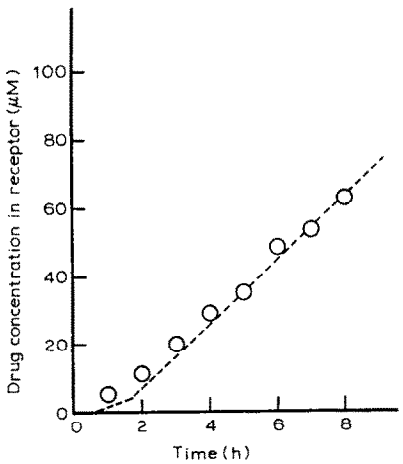


Fig. 4. Percutaneous permeation of 5-fluorouracil through rat skin in vitro after its coapplication with 1-octylcarbamoyl-5-fluorouracil. Each point is the mean of two experiments. Dashed line represents sum of appearance of **I** after application of **I** and that after application of **IV**.

phobic stratum corneum but that the more lipid-soluble compound does not diffuse adequately through the stratum corneum. The present study has demonstrated the usefulness of the prodrug approach for optimal transdermal delivery.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. We wish to thank Keiko Sugamoto and Emi Sato for skilled technical assistance. We thank Mitsui Pharmaceuticals, Inc., for supplying compounds **I**, **III** and **IV**.

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