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Pro-drugs as drug delivery systems XXIII. Improved dermal delivery of 5-fluorouracil through human skin via N-acyloxymethyl pro-drug derivatives

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Summary

The percutaneous permeation characteristics of two N-1-acyloxymethyl derivatives of 5-fluorouracil were determined and compared with that of 5-fluorouracil using excised human skin mounted in open diffusion cells. The derivatives, particularly 1-butyryloxymethyl-5-fluorouracil, permeated more readily through the human skin than 5-fluorouracil and at the same time were delivered in the form of parent drug due to cutaneous metabolism as mediated by hydrolytic enzymes. The better permeabilities of the derivatives were ascribed to their higher lipophilicities as expressed in terms of the octanol–water partition coefficients. It appeared that N-acyloxymethyl derivatives of 5-fluorouracil may be promising pro-drug candidates with enhanced topical bioavailability compared to the parent drug. Leaching of hydrolytic enzymes from the skin preparations into the receptor phase was found to take place during the permeation study and its significance is discussed in relation to the question of extent of cutaneous and bulk phase metabolism.

Introduction

Topical application of 5-fluorouracil has proved to be a valuable treatment of various diseases including actinic keratoses, various epithelial neoplasms and psoriasis (Dillaha et al., 1963; Waldorf et al., 1966; Klein et al., 1970; Tsuji and Sugai, 1972; Cullen, 1979). The drug is most often administered topically in the form of 1–5% solutions in propylene glycol since this vehicle is known to effectively increase the skin penetration of various topical drugs (Lorenzetti, 1979).

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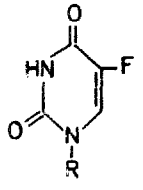
Although being a valuable topical agent and, to some extent, being capable of permeating excised human skin (Cohen and Stoughton, 1974) 5-fluorouracil appears to have undesirable physical properties such as low lipophilicity (described later) for achievement of optimal topical bioavailability. A promising approach to enhance the topical absorption of polar drugs may be development of lipophilic transient derivatives (pro-drugs) which after diffusion into and/or through the skin undergo reconversion into the parent active drug molecules. The use of this approach for improving the dermal delivery of various drugs including vidarabine (Fox et al., 1979; Yu et al., 1979a and b, 1980a and b), cromolyn (Bodor et al., 1980) and acetylsalicylic acid (Loftsson and Bodor, 1981) has been the subject of recent investigations but it is still in its infancy (Stella et al., 1980).

The objective of the present work is to test the percutaneous permeation characteristics of two N-1-acyloxymethyl derivatives (II and III) of 5-fluorouracil (I) in comparison with the parent drug. These derivatives were chosen in the belief that they have a higher lipophilicity than 5-fluorouracil hence possessing a higher stratum corneum permeability, and that they may be susceptible to undergoing reconversion into the parent drug in the skin by virtue of enzyme-mediated hydrolysis. In the permeation study excised human skin mounted in open diffusion cells was used. This in vitro technique has been found capable of differentiating various compounds with respect to skin permeability and of ranking them in an order that parallels that seen in vivo (Franz, 1975).



I R = −H

II R = $-CH_2OC(=O)CH_2CH_2CH_3$

III R = $-CH_2OC(=O)C(CH_3)_3$

Materials and methods

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer, using 1 cm cuvettes. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. High-performance liquid chromatography (HPLC) was done with a Spectra-Physics model 3500B instrument equipped with a 10 μl loop injection valve. The column used, 25 cm long and 4.6 mm i.d., was packed with Nucleosil C-18 (10 μm particles).

Chemicals

5-Fluorouracil was purchased from Sigma, St. Louis. The N-acyloxymethyl derivatives of 5-fluorouracil (II and III) were prepared as described elsewhere (Johansen et al., 1982). Buffer substances and all other chemicals or solvents used were of reagent grade.

Analysis of 5-fluorouracil and its pro-drugs

An HPLC method was used to analyze the receptor phase samples or aqueous buffer samples for 5-fluorouracil and pro-drug content. For analysis of 5-fluorouracil the reversed-phase column was eluted isocratically at ambient temperature with a mobile phase consisting of 0.01 M acetate buffer pH 4.0–methanol (93:7 v/v). The flow rate was 1.6 ml · min^{-1} and the column effluent was monitored at 266 nm. Under these conditions 5-fluorouracil showed a retention time of 2.2 min whereas the ester derivatives II and III were retained on the column for more than 20 min.

The chromatographic determination of the esters II and III was done with a mobile phase of 0.01 M acetate buffer pH 4.0–methanol (47:53 v/v), the other chromatographic conditions being the same as described above. Ester II showed a retention time of 3.1 min and ester III 3.9 min whereas 5-fluorouracil eluted almost with the solvent front. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards (prepared in water with 5% ethanol) chromatographed under the same conditions. The detection limit was 0.1 $\mu g \cdot ml^{-1}$.

It was confirmed that substances leached from the skin samples into the receptor phase showed no interferences with the peaks of 5-fluorouracil and the esters.

Hydrolysis of 5-fluorouracil pro-drugs

The rate of hydrolysis of the ester derivatives II and III in 0.05 M phosphate buffer solution, pH 7.24 (pure receptor phase), as well as in a receptor phase sample which had been exposed to a skin preparation for 90 h was determined using the HPLC procedure for 5-fluorouracil quantitation. Accurately weighed samples of the esters (about 8 mg) were dissolved in 0.25 ml of ethanol and 10.00 ml of buffer solution or exposed receptor phase pre-equilibrated at 37°C were added. The solutions were kept at 37°C in a water-bath and at suitable intervals 500 μl aliquots were removed, diluted 10-fold with water and analyzed for 5-fluorouracil. The initial rates of 5-fluorouracil formation were determined from the slopes of linear plots of amount of 5-fluorouracil released vs time. Dividing these rates by the initial concentration of ester derivatives afforded pseudo-first-order rate constants for the hydrolysis. In the runs with the ester derivative II analysis was also made of undegraded ester. In these cases pseudo-first-order rate constants were obtained from plots of the logarithm of remaining ester against time. The rate constants obtained using these methods agreed within ±5%.

Measurement of aqueous solubility and partition coefficients

The solubility of 5-fluorouracil and the ester derivatives II and III in water was determined at 22°C by placing excess amounts in 10 ml of water. The mixtures were rotated for 24 h and filtered. The concentrations of the compounds in their saturated solutions were determined spectrophotometrically at 266 nm. Analysis by HPLC showed insignificant degradation of the esters during the solubility determination.

The partition coefficients of the compounds were determined in a 1-octanol–water system as previously described (Bundgaard et al., 1979). The aqueous phase was either water or 0.1 M Tris buffer, pH 7.40. The initial concentrations of the

compounds in the aqueous phase were about 2×10^{-4} M. The solute concentrations in this phase before and after partitioning were determined spectrophotometrically at 266 nm.

Permeability–metabolism studies using excised human skin

Whole abdominal human skin obtained under autopsy from a single donor was used. The skin was stored at −18°C and was allowed to thaw gradually at room temperature before use. All subcutaneous fat was removed carefully and the skin was cut into 6 pieces. The excised skin was then mounted in open diffusion cells of the same type as those used by Franz (1975) and having an available diffusion area of 1.8 cm^2. The dermal side of the skin was exposed to the receptor medium (7.5 ml of 0.05 M isotonic phosphate buffer solution of pH 7.2) and each cell was placed on a magnetic stirrer in a 37°C incubator.

Solutions of the test compounds were made in ethanol containing 12% propylene glycol. Then 50 μl of this solution (corresponding to 200 μg of the test compounds) was applied per cm^2 to the skin samples. The ethanol evaporated spontaneously within a few minutes, leaving a solution of the compounds in propylene glycol spread uniformly on the skin surface. At appropriate intervals samples of 2 ml were removed from the receptor phase and replaced with fresh buffer. The samples were stored in screw cap tubes at −20°C until they were analyzed for 5-fluorouracil and pro-drug content by HPLC as described above. The permeation studies of each compound were done in duplicate, the data obtained being reproducible within ±15%.

Results and discussion

The obtained permeation profiles for 5-fluorouracil and the ester derivatives II and III in terms of percentage in the receptor phase of the total amount applied to the excised human skin as a function of time are shown in Figs. 1 and 2. The times required for the substances to reach steady-state diffusion in the skin can be seen to be 15–20 h. The profiles are all curvilinear and reveal two steady-state regions which are characterized by a drop in rate of permeation after about 45 h. This behaviour indicates significant time-dependent changes in the interplay of forces governing the permeation of the compounds across the skin. A plausible explanation may be that the vehicle gradually diffuses through the skin, thereby causing a change in the thermodynamic activity of the compounds in the donor phase. Turi et al. (1979) have found that propylene glycol easily permeates the skin, and their work suggests a flux of the solvent of about 1 $mg \cdot h^{-1} \cdot cm^{-2}$ in hairless mouse skin. In the present study the amount applied of propylene glycol is 6 $mg \cdot cm^{-2}$.

The comparative cumulative permeation profiles show that the 1-butyryl-oxymethyl derivative (II) of 5-fluorouracil penetrates the skin at a rate about 5 times faster than that of 5-fluorouracil, while the 1-pivaloyloxymethyl derivative (III) is absorbed at a rate about 2 times faster. These differences in skin permeability rest certainly on the different physicochemical characteristics of the compounds. As seen

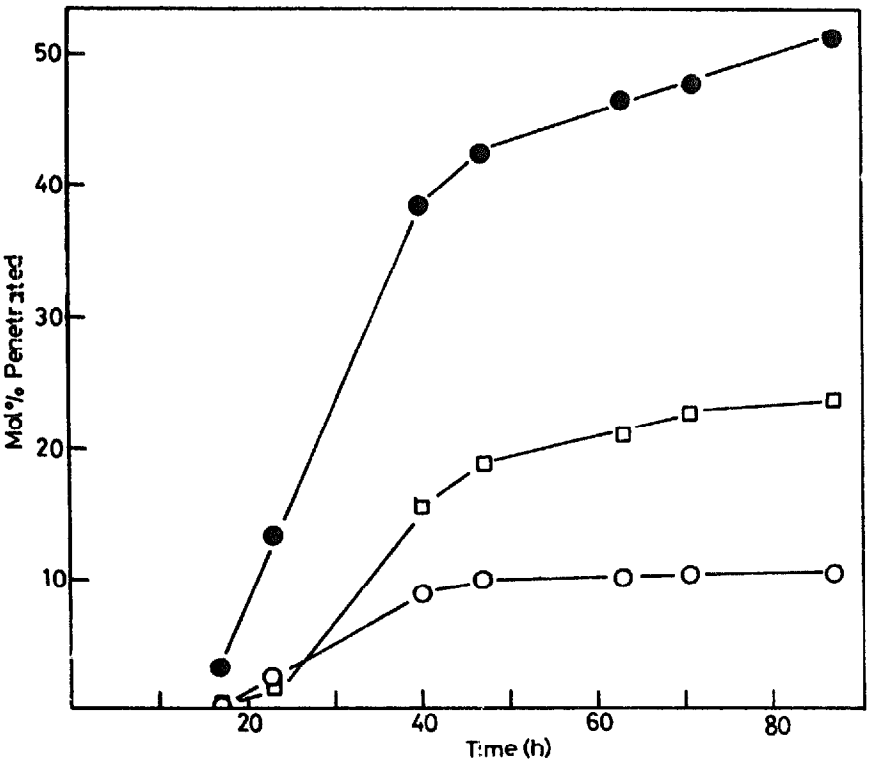


Fig. 1. Permeability of 5-fluorouracil (I), 1-butyryloxymethyl-5-fluorouracil (II) and 1-pivaloyloxymethyl-5-fluorouracil (III) through human skin as mol percentage appearing in the receptor phase relative to the total amount applied to the skin. Key: ○, I from I applied; ●, I from II applied; and □, sum of I and III from III applied.

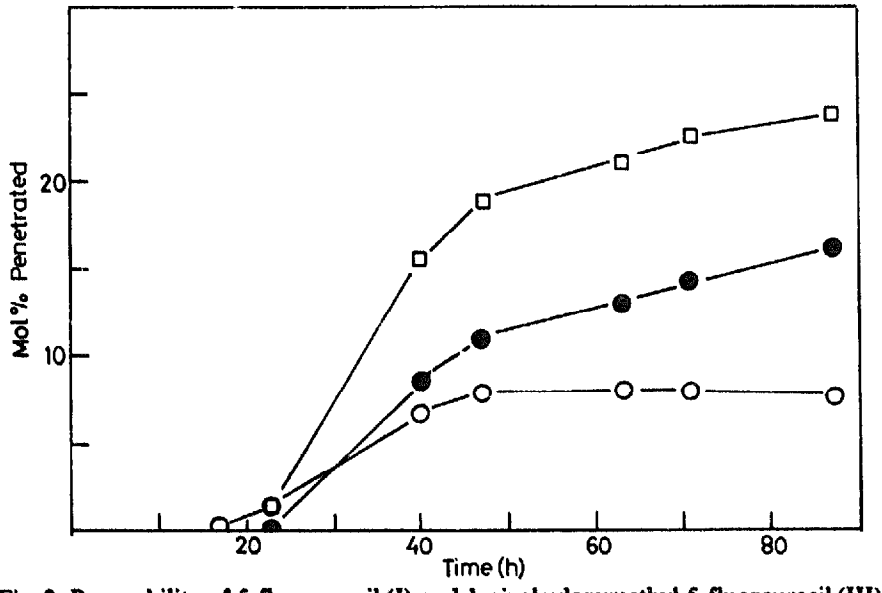


Fig. 2. Permeability of 5-fluorouracil (I) and 1-pivaloyloxymethyl-5-fluorouracil (III) through human skin as mol percentage appearing in the receptor phase relative to the total amount applied to the skin. Key: ○, III from III applied; ●, I from III applied; and □, sum of I and III from III applied.

TABLE 1

PARTITION COEFFICIENTS AND AQUEOUS SOLUBILITIES OF 5-FLUOROURACIL AND THE DERIVATIVES II–III

Compound	log P [a]	S [b] ($mg \cdot ml^{-1}$)
5-Fluorouracil (I)	−1.00	12.2
1-Butyryloxymethyl-5-fluorouracil (II)	0.38	11.4
1-Pivaloyloxymethyl-5-fluorouracil (III)	0.66	2.5

[a] Between 1-octanol and water at 22°C.
[b] The solubility in water at 22°C.

from Table 1 the derivatives II and III are more lipophilic than 5-fluorouracil as expressed in terms of partition coefficients between octanol and water and because of the increased lipophilicity, the derivatives might penetrate the lipoidal stratum corneum more readily. Although having the highest lipophilicity, however, the derivative III showed a lower rate of permeation than compound II which means that factors other than lipophilicity are important, e.g., drug–vehicle interactions.

It is interesting to note that despite the greatly increased lipophilicity the derivatives II and III possess only slightly lower aqueous solubility than 5-fluorouracil (Table 1). The relatively poor solubility of uracil in both water and organic solvents is largely a result of the high crystal lattice energy in the molecule due to intermolecular hydrogen bonds formed between NH-protons in one molecule and a carbonyl group in another molecule (Bansal et al., 1981). Disruption or decrease of such hydrogen bonding by replacement of the N-1 of N-3 protons in uracil by methyl groups results in greatly increased solubility (Bansal et al., 1981) and the behaviour of 5-fluorouracil and its N-1 derivatives may be similarly explained.

In the permeation experiments with compound II no unchanged derivative was detected in the receptor phase (i.e less than 0.1 $\mu g \cdot ml^{-1}$) during the study (Fig. 1) whereas in the case of compound III both unchanged derivative and 5-fluorouracil were quantitated in the receptor phase (Fig. 2). To determine whether the hydrolytic cleavage of the derivatives II and III to yield 5-fluorouracil took place during the diffusion of the compounds through the skin or when the compounds were in the receptor phase the stability of the compounds in this phase was investigated. Using an HPLC procedure the hydrolysis of the derivatives with formation of 5-fluorouracil in stoichiometric amounts was found to follow first-order kinetics with the rate constants listed in Table 2. The rate of formation of 5-fluorouracil is quite low in the phosphate buffer, the half-lives being several days. The hydrolysis was, however, found to proceed 3–5 times faster when the receptor phase used in the stability study had been in contact with the skin sample for 90 h under conditions similar to those used in the permeation study (except that no aliquot samples were withdrawn during the period concerned). The data obtained are included in Table 2, and Fig. 3 shows plots of the percentage of 1-butyryloxymethyl-5-fluorouracil remaining as a function of time during incubation of the compound in exposed-to-skin

TABLE 2

PSEUDO-FIRST-ORDER RATE CONSTANTS (k) AND HALF-LIVES ($t_{1/2}$) OF HYDROLYSIS OF THE ESTER DERIVATIVES II AND III TO 5-FLUOROURACIL AT pH 7.24 (0.05 M PHOSPHATE BUFFER) AND 37°C

Compound	Buffer		Buffer exposed to skin sample for 90 h	
	$k(h^{-1})$	$t_{1/2}(h)$	$k(h^{-1})$	$t_{1/2}(h)$
II	0.0050	139	0.022	32
III	0.0011	630	0.0033	210

and non-exposed receptor phase. Although the hydrolysis is accelerated in the former phase the stability data in conjunction with the permeation profiles in Fig. 1 clearly show that the inability to detect undegraded compound II in the receptor phase is not due to loss of the compound by degradation in this phase, but instead implies that the conversion of the compound to 5-fluorouracil indeed occurs in the skin. It is well known that whole skin, particularly the epidermis, contains many highly active enzyme systems including esterases (Pannatier et al., 1978, 1981; O'Neill and Carless, 1980; Yu et al., 1980a and b) and previous studies using hairless mouse skin preparations have described similar simultaneous skin transport and esterase-mediated metabolism of pro-drugs (Yu et al., 1979a and b, 1980a and b; Bodor et al., 1980; Loftsson and Bodor, 1981).

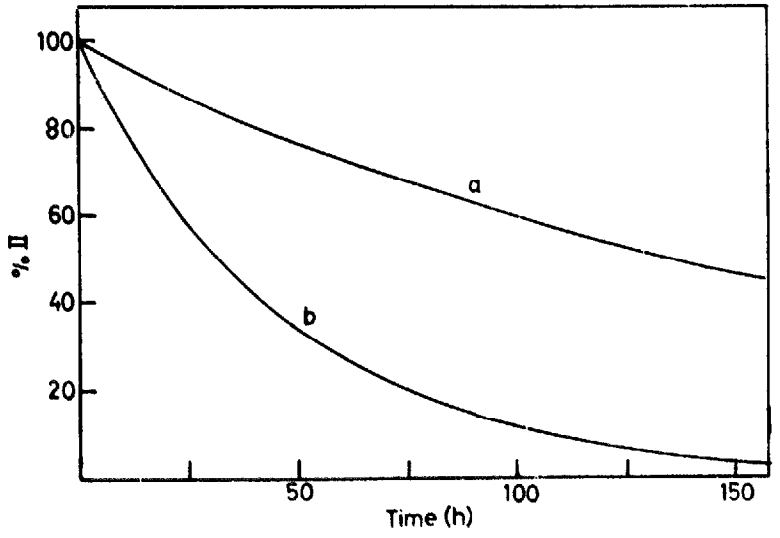
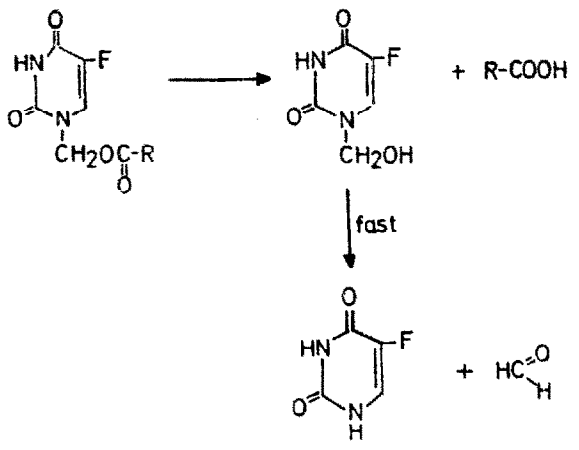


Fig. 3. Time-courses of degradation of 1-butyryloxymethyl-5-fluorouracil at 37°C in the receptor phase (a) and in receptor phase that had been kept in contact with human skin in a diffusion cell for 90 h (b) The curves were calculated from the rate constants given in Table 2.



Scheme I

The hydrolysis of the N-acyloxymethyl derivatives is assumed to take place as depicted in Scheme I. Enzymatic cleavage of the ester grouping results in the formation of 1-hydroxymethyl-5-fluorouracil which is decomposed instantaneously into formaldehyde and 5-fluorouracil in accord with the behaviour of other similar N-hydroxymethyl derivatives (Johansen and Bundgaard, 1979, 1981; Bundgaard and Johansen, 1980; Bansal et al., 1981). The suggested mechanism of hydrolysis is supported by the finding that the rates of formation of formaldehyde are identical to those of formation of 5-fluorouracil and to those of N-acyloxymethyl derivative disappearance as determined in neutral buffer or plasma reaction solutions (Johansen et al., 1982).

The results obtained clearly establish cutaneous metabolism to be predominantly responsible for the conversion of the derivatives II and III into 5-fluorouracil. The pivaloyloxymethyl derivative is more resistant to undergoing spontaneous as well as enzyme-mediated cleavage than the less sterically hindered butyryloxymethyl derivative (Table 2) and it appeared in the receptor phase both in unchanged form and in the form of parent drug.

Leaching of hydrolytic enzymes, most probably esterases, from human skin samples in the permeation experiments have apparently not been reported before but it finds some recent precedents in studies using hairless mouse skin. Thus, Ando et al. (1977) and Yu et al. (1979b) showed that adenosine deaminase, an enzyme that metabolizes the antiviral drug vidarabine into 9-β-D-arabinofuranosylhypoxanthine, rapidly leaches out of the mouse skin from the dermis side into the receptor phase compartment of the permeation model apparatus, the leaching being complete after a period of about 6 h. Yu et al. (1980b) also found that esterase enzymes capable of hydrolyzing the 5′-valerate ester of vidarabine leach out from the dermis side of a hairless mouse skin preparation and that the leaching was essentially completed within the first 2 h. These findings along with the present observation for human skin preparations emphasize the need for considering receptor phase drug metabolism due to leached enzymes when studying concurrent transport and metabolism of drugs in the skin. Some studies in this area, as e.g. those by Loftsson and Bodor

(1981) and Loftsson (1982), on the percutaneous absorption and metabolism of acetylsalicylic acid and pro-drugs thereof have apparently not taken the possible leaching of enzymes into account when assessing the stability of the compounds in the receptor phase.

The receptor phase hydrolysis of the compounds II and III due to leached enzymes was only determined after 90 h of leaching. The rate of leaching remains to be investigated but on basis of the studies cited above using mouse skin preparations the leaching may have been completed long before 90 h.

In conclusion, the results of this study demonstrate that N-acyloxylmethyl derivatives of 5-fluorouracil may be promising pro-drug candidates with the purpose of providing a more efficient topical delivery of the parent drug. This conclusion is especially evident on the basis of the results obtained with the butyryloxymethyl derivative in that this compound permeates about 5 times more readily through the human skin than 5-fluorouracil and at the same time is delivered completely in the form of the parent drug due to extensive cutaneous metabolism.

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