International Journal of Pharmaceutics, 36 (1989) 43–50
Elsevier

IJP 01889

Urea analogues in propylene glycol as penetration enhancers in human skin

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(Received 20 April 1989)
(Accepted 10 May 1989)

Key words: Percutaneous absorption; Penetration enhancer; Urea; Urea analogue; Propylene glycol; 5-Fluorouracil

Summary

Urea, 1-dodecylurea, 1,3-didodecylurea and 1,3-diphenylurea were assessed as skin penetration enhancers for the model penetrant 5-fluorouracil (5-FU). The permeability coefficient (K_p) was determined for 5-FU applied in saturated aqueous solutions to human epidermal membranes. Then each urea was applied as a saturated solution in dimethylisosorbide, light liquid paraffin or propylene glycol: the solutions were removed and K_p was redetermined; the enhancement ratio (K_p after enhancer treatment/K_p before enhancer treatment) measured the accelerant effect. Urea and the vehicles alone were ineffective as enhancers; the urea analogues behaved similarly at saturation in any one vehicle; and the analogues were only effective when delivered from propylene glycol, enhancing the permeation of 5-FU 6 times by increasing the diffusivity of the stratum corneum. Thus, the role of propylene glycol as a synergistic vehicle for penetration enhancers was confirmed.

Introduction

Topical administration of therapeutic agents promises many advantages over oral and intravenous administration (Barry, 1983; Guy and Hadgraft, 1985). However, the relative impermeability of the stratum corneum offers considerable resistance to drug permeation. In attempts to reduce reversibly this diffusional barrier, researchers have employed penetration enhancers (or accelerants) which interact with stratum corneum constituents, disrupting the highly ordered structure (e.g. Southwell and Barry, 1983; Barry et al., 1984; Southwell and Barry, 1984; Goodman and Barry, 1988; Okamoto et al., 1988). Ideally, a penetration enhancer is pharmacologically inert, has a specific, immediate yet reversible, action and is cosmetically acceptable (Barry, 1983; Hadgraft, 1984; Woodford and Barry, 1986). In the present study, urea and 3 analogues, dissolved in 3 vehicles, were compared for their penetration-enhancing activities towards the cytotoxic agent 5-fluorouracil (5-FU), chosen as a model penetrant.

Urea is a mild keratolytic agent used in the treatment of ichthyosis and other hyperkeratotic skin conditions. As a 10% cream, it increases the water-holding capacity of the stratum corneum by 100%, and has little effect on the epidermal water barrier (Grice et al., 1973). The moisturizing and

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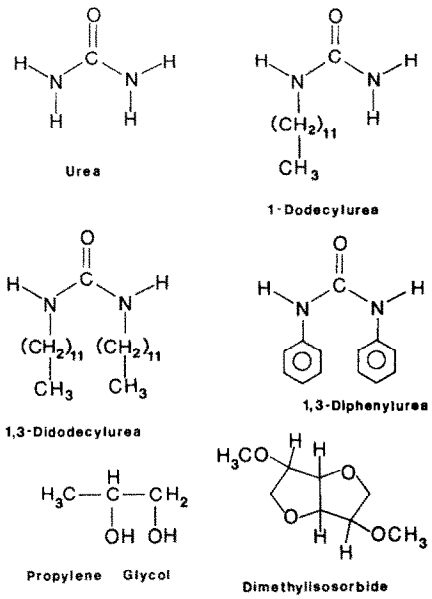

Fig. 1. The structural formulae of the urea analogues and vehicles evaluated as penetration enhancers.

keratolytic effects of urea increase the activity and bioavailability of hydrocortisone from Alphaderm cream (Barry and Woodford, 1977; Barry, 1983; Woodford and Barry, 1984). As a 10% solution in propylene glycol (PG), urea has no effect on naloxone flux through human cadaver skin (Aungst et al., 1986).

Established penetration enhancers such as Azone, which contains a C12 saturated hydrocarbon chain, interact with and disrupt the structured lipid environment in the stratum corneum (Barry, 1987a and b; Goodman and Barry, 1989). We investigated the possibility of combining the moisturizing and keratolytic properties of urea with the disrupting effects of alkyl and aryl groups, one or two per molecule. The chemicals thus tested for penetration-enhancing activity towards 5-FU were: urea, 1-dodecylurea (DDU), 1,3-didodecylurea (DDDU) and 1,3-diphenylurea (DPU); the vehicles were dimenthylisosorbide (DMI), light liquid paraffin (LLP) and (PG) (Fig. 1).

DMI, promoted for use in cosmetics, is a solvent which is poorly adsorbed by the skin and appears to have little penetration-enhancing activity (Barry et al., 1984; Bennett et al., 1985). It has therefore been selected as a standard vehicle for comparisons of potential penetration enhancers. Light liquid paraffin is a widely used lipophilic vehicle for topical preparations. It is used as an emollient in irritant skin conditions and for the removal of desquamative crusts. PG is valuable in dermatological formulations and as a cosolvent for penetration enhancers. It has been reported to increase the permeation of oestradiol (Mollgaard and Hoelgaard, 1983a) and hydrocortisone (Barry and Bennett, 1987) through excised human abdominal skin, yet is ineffective in promoting the topical bioavailability of betamethasone 17-benzoate as assessed by the occluded vasoconstrictor assay (Barry et al., 1984). It is also ineffective in promoting permeation of metronidazole through excised full-thickness human skin (Mollgaard et al., 1988) and PG pretreatment of human epidermal membranes has no significant effect on 5-FU pseudo-steady state permeation (Goodman and Barry, 1988). However, when used in combination with accelerants such as Azone and oleic acid, PG shows a marked synergistic response (e.g. Barry and Bennett, 1987; Barry, 1987a; Goodman and Barry, 1988).

Materials and Methods

Urea and DPU (Sigma Chemical Company) were used as received; DDU and DDDU were synthesised (Erickson, 1954). PG (B.D.H. Chemicals Ltd.), DMI (Aldrich Chemical Company) and LLP (B.D.H. Chemicals Ltd.) were used as supplied. 5-[6-^{3}H]FU (Amersham International PLC) was the model permeant, a saturated aqueous solution (10.2 mg/ml at $32 \pm 1°$C; Bond and Barry, 1988) being prepared with the help of unlabelled 5-FU (Sigma Chemical Company). Saturated solutions of urea and the analogues were prepared in the 3 vehicles, the approximate concentrations being evaluated gravimetrically. Partition coefficients (octanol/water) of urea and the analogues were calculated by the fragment method of Hansch and Leo (1979).

Synthesis

1-Dodecylurea. A mixture of dodecylamine (18.5 g, 0.10 mol), urea (6.6 g, 0.11 mol) and

pyridine (200 ml) was refluxed for 4.5 h in a fume cupboard, cooled over ice and the crystalline product filtered off under suction. The crystals were washed with water to remove excess urea and pyridine. The product was recrystallised from chloroform to a constant melting point, determined by differential scanning calorimetry (Perkin-Elmer 7 Series Thermal Analysis System) of 107.2° C. The literature gives 106.8–107.5° C (Erickson, 1954). The thermal analysis showed the product to be approximately 98% pure.

1,3-Didodecylurea. A mixture of dodecylamine (13.0 g, 0.07 mol), urea (2.0 g, 0.03 mol) and butan-1-ol (20 ml) was refluxed for 30 h in a fume cupboard and cooled over ice and the crystalline product was filtered off under suction. Recrystallisation from acetone gave a constant melting point of 104.8° C, and a purity of approximately 92%, which is adequate for penetration enhancer studies. The literature gives 103.3–105.5° C (Erickson, 1954).

Preparation of human epidermal membranes

Caucasian abdominal skin (male and female, 70–89 y) obtained post-mortem was stored frozen at −20° C (Harrison et al., 1984). Epidermal membranes were prepared by the heat separation technique of Kligman and Christophers (1963). Excess fatty and connective tissues were removed from the skin which was then immersed in water at 60° C for 45 s. The epidermal membrane was teased off the underlying dermis and floated on an aqueous solution of 0.002% sodium azide for 36 h to ensure that the stratum corneum was fully hydrated.

Permeation experiments

Experiments at 32 ± 1° C used an automated diffusion apparatus with 24 stainless-steel diffusion cells, diffusional area 0.126 cm^2, and 0.002% aqueous sodium azide receptor solution (Akhter et al., 1984). Fully hydrated epidermal membrane samples were mounted in the cells and 150 µl aliquots of saturated, radiolabelled 5-FU solution placed in the donor compartments which were covered. 4 ml samples of receptor solution were collected every 2 h for 36 h, to which 10 ml Scintran Cocktail T was added, and the radiolabelled drug determined by liquid scintillation counting (Packard 460C). The permeant solution was washed from the membrane with 0.002% sodium azide solution and replaced with 150 µl saturated solution of urea or an analogue in one of the vehicles. After 12 h the test solution was washed from the membrane and the permeation of radiolabelled 5-FU again monitored for 36 h.

Partitioning experiments

The effect of urea analogue/PG formulations on the partitioning of 5-FU was investigated. Permeation of 5-FU through untreated epidermal membranes was monitored, and at pseudo steady state flux the concentration of the drug in the membrane was determined as follows. The epidermal membranes were removed from the diffusion cells, rinsed with distilled water, blotted dry and the diffusional areas were solubilised in 1 ml Soluene-350. 10 ml Scintran Cocktail T scintillation fluid and 0.1 ml glacial acetic acid were added and samples stored at room temperature overnight to allow chemiluminescence to subside. Acidification of the mixture reduces non-radiation events which may interfere with drug determination. The concentration of 5-FU in the membrane was evaluated by liquid scintillation counting. The pseudo-steady-state concentration of 5-FU in epidermal membranes after 12 h treatments with a urea analogue/PG mixture were similarly determined to illustrate the accelerant effects on partitioning of the drug into the tissue.

Results and Discussion

Example permeation profiles of the drug through the membrane before and after treatment with a solution of the urea analogues saturated in propylene glycol are given in Fig. 2. Computer-aided analysis of these results evaluated the permeability coefficient (K_p) of the drug in the membrane before and after treatment with a penetration enhancing solution. A measure of the penetration-enhancing activity of the agent, the enhancement ratio (E.R.), may be calculated

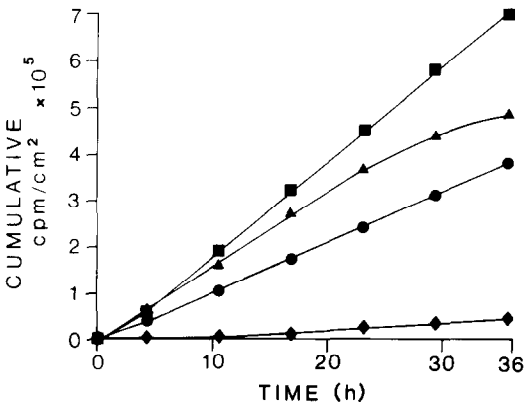

Fig. 2. Example permeation profiles of 5-FU through human epidermal membranes before and after treatment with saturated solutions of the urea analogues in propylene glycol: squares, DDU; triangles, DPU; circles, DDDU; diamonds, control.

(Goodman and Barry, 1988):

E.R. = K_p of membrane after application of penetration enhancer/ K_p of membrane before application of penetration enhancer

The values reported were the mean enhancement ratios from a minimum of 5 replicates.

The experimental design of determining K_p, treating the epidermal membrane with penetration enhancers, and then redetermining K_p, allows each piece of skin to act as its own control, thereby reducing errors due to the biological variability of human skin. The conditions for drug delivery were maximised with the use of saturated drug solutions, thereby maintaining the permeant at its maximum thermodynamic activity. The epidermal membrane was fully hydrated, a condition which enhances the permeation of most penetrants, including 5-FU (Barry, 1987a; Goodman and Barry, 1989). This last condition thus provides a stringent test of penetration enhancing activity. The use of saturated solutions of urea and its analogues allows a direct comparison of the penetration-enhancing abilities of each agent from different vehicles as the chemical potential of the penetration enhancer is constant (maximal) in all the test solutions. The approximate saturated concentrations of the test agents in the different vehicles,

TABLE 1

The approximate saturated concentrations (mg/ml) of urea and the analogues in the 3 vehicles at room temperature (19 ± 1 °C), and the calculated log partition coefficients (octanol/water) for urea and the analogues

Urea analogue	Vehicle			log P
	LLP	DMI	PG	
DDU	0.3	3.8	2.6	4.77
DDDU	0.2	0.6	0.7	11.7
DPU	1.0	1.6	15	2.98
Urea	1.6	5.0	27	−2.11

and the calculated log partition coefficient (log P) values, are give in Table 1. All the test agents are poorly soluble in LLP with urea having the greatest concentration of 1.6 mg/ml. The saturated concentrations of DDU and DDDU in DMI and PG are similar, thus any differences observed in the penetration-enhancing effects of these analogues from the two vehicles is unlikely to be due to a large difference in concentrations of the test agents.

A comparison of the permeability coefficients of the penetration enhancers from the 3 vehicles is given in Table 2. From these results, the mean control value for the permeability coefficient of 5-FU in the untreated membrane at 32 °C is 2.16

TABLE 2

Mean permeability coefficients of 5-FU through human cadaver skin, with standard error of the mean, before and after treatment with urea analogues applied from 3 vehicles: a, before treatment; b, after treatment

Urea analogue		Permeability coefficient $\times 10^5$ (cm/h)		
		LLP	DMI	PG
Vehicle alone	a	2.17 ± 0.30	1.84 ± 0.98	3.36 ± 1.24
	b	2.60 ± 0.63	1.73 ± 0.47	4.18 ± 1.87
Urea	a	2.38 ± 0.74	3.48 ± 1.36	1.56 ± 0.36
	b	1.57 ± 0.33	3.50 ± 1.44	1.71 ± 0.46
DDU	a	3.74 ± 1.07	0.93 ± 0.27	0.96 ± 0.25
	b	2.72 ± 0.93	2.03 ± 0.96	4.17 ± 1.15
DDDU	a	3.28 ± 0.64	0.58 ± 0.10	1.24 ± 0.18
	b	2.57 ± 0.70	1.25 ± 0.36	3.49 ± 0.77
DPU	a	5.30 ± 1.39	0.79 ± 0.22	0.77 ± 0.05
	b	5.68 ± 3.01	0.79 ± 0.07	2.67 ± 0.33

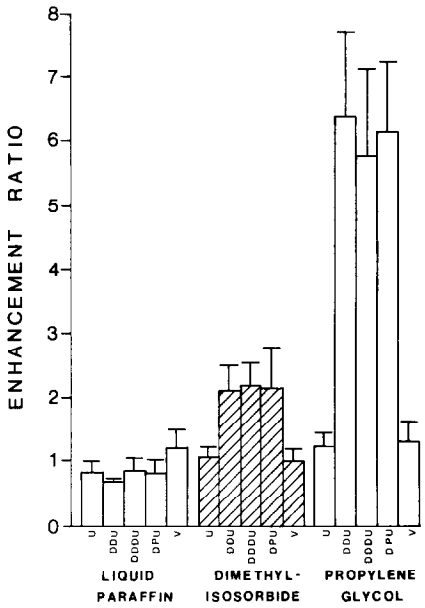

Fig. 3. The mean enhancement ratios of urea and the analogues from the 3 vehicles, with S.E.M.; u, urea; V, vehicle alone.

$\pm 0.35 \times 10^{-5}$ cm/h ($n = 75$), a value that shows good agreement with other published data (Goodman and Barry, 1988). The activity of the urea analogues are more clearly demonstrated in terms of the enhancement ratios, the mean values of which are shown in Fig. 3. These results show that the vehicles alone, and urea saturated in the vehicles, produce no significant increase in the permeability coefficient of 5-FU ($P = 0.05$). Also, no significant difference exists in the penetration-enhancing activities of the three urea analogues delivered from a given vehicle ($P = 0.05$). However, the choice of vehicle clearly affects the enhancing activity of the test agents. In particular, when applied as a saturated solution in propylene glycol, the enhancement ratios of the urea analogues are significantly greater than when applied saturated in DMI or LLP ($P = 0.05$).

The mechanisms of action of penetration enhancers are becoming clear, and a general theory of accelerant activity based on molecular changes in the stratum corneum has been proposed (Barry, 1987a; Goodman and Barry, 1989). Based on this theory, penetration enhancers may act mainly by one or more of 3 main mechanisms; disruption of the highly ordered lipid structure between the corneocytes, interaction with intracellular proteins, and partitioning effects, a concept formalised as the lipid–protein partitioning (LPP) theory (Barry, 1989).

The synergistic effect of PG with a variety of penetration enhancers such as Azone and oleic acid is well documented (Cooper, 1984; Sheth et al., 1986; Barry, 1987a), and studies by Wotten et al. (1985) concluded that the glycol is necessary to maximise the penetration-enhancing properties of Azone. Differential scanning calorimetry studies of PG-treated stratum corneum show an alteration in the intracellular keratin structure, probably due to displacement of bound water (Goodman and Barry, 1989). This effect reduces drug/skin binding, thereby enhancing intracellular transport. However, this effect would only be important under conditions whereby the intercellular lipid structures were not rate-limiting in diffusion, or had been disrupted by a penetration enhancer (Barry, 1987a). PG permeates the skin in substantial amounts (Mollgaard and Hoelgaard, 1983b). With urea analogue PG mixtures, the glycol permeating into the skin will enhance partitioning of the lipophilic accelerants into the stratum corneum. Once in the lipoidal environment, the hydrophobic moieties of the penetration enhancers may interact to disrupt the highly ordered barrier structure. Fig. 2 shows a reduction in the lag time (L) for 5-FU permeation after treatment with each urea analogue in PG. The lag time is related to the diffusivity (D) of the drug in the membrane by:

$$L = \frac{h^2}{6D}$$

where h is the membrane thickness (taken as approximately 3×10^{-3} cm for human abdominal stratum corneum). Thus the diffusivity of 5-FU in the membrane after treatment with DDU in PG (mean log time 1.03 h, $n = 4$) may be calculated approximately:

$$D = \frac{h^2}{6L} = 1.46 \times 10^{-6} \text{ cm}^2/\text{h}$$

Comparing this with the diffusivity of the membrane prior to treatment (mean lag time 9.65 h, $n = 17$) of 1.55×10^{-7} cm^2/h, shows a 9.4-fold increase in diffusivity after treatment with the urea analogue. It is widely accepted that many molecules traversing the stratum corneum do so by a tortuous intercellular pathway, and the diffusional pathlength for a molecule has recently been speculated to be approximately 350 μm (Guy and Hadgraft, 1988). Thus, the value of h used above to calculate D may be significantly underestimated. However, the precise value for diffusional pathlength is irrelevant when taking the ratio of diffusivities before and after enhancer treatment, as the pathlength is assumed to be constant in both cases. This may not be the true situation as PG may alter the epidermal membrane thickness. Thus the diffusivity values, and their ratios, are approximate, but are useful guides to molecular events within the tissue.

The 9-fold increase in diffusivity correlates with a mechanism of action whereby the penetration enhancer disrupts the lipid structure of the stratum corneum. The partition coefficient (P) of the drug from its vehicle (aqueous solution) into the stratum corneum is related to the membrane diffusivity by:

$$P = \frac{K_p \cdot h}{D}$$

Thus, the partition coefficient for the drug may be evaluated approximately to give a control pretreatment value of 0.484 and a posttreatment value of 0.327. As expected, the presence of PG and the urea analogue in the membrane correlates with a reduction in partitioning of the drug into the tissue, by a factor of 0.68. To verify this conclusion, the steady state concentrations of 5-FU in epidermal membranes were determined. After treatment with DDU in PG the steady state concentration of 5-FU in the membrane fell by a factor of 0.71 ± 0.09 ($n = 3$), which is in good agreement with the factor of 0.68 above. Therefore, we conclude that the experimentally determined enhancement ratio with DDU in PG is composed of an increase in diffusivity of the membrane and a decrease in partitioning of the drug, which give a combined effect: $9.42 \times 0.68 = 6.40$ (= E.R.).

A similarly reduced lag time was observed with DDDU and DPU, again illustrating increased membrane diffusivity and reduced drug partitioning.

The calculated log P (octanol/water) values for the urea analogues range from approximately 3 to 11.7, and we expect the rank-order of the analogues in this system to correlate with log P (stratum corneum/water). Clearly, with PG present in the stratum corneum, the solvent nature of the membrane is altered and hence the partition coefficient data are of little value for predicting the amounts of the accelerants entering the membrane. However, once the analogues are in the lipid domain of the stratum corneum, their clearance into aqueous receptor solutions will be governed by a partitioning mechanism. DDDU (log $P = 11.7$) is not cleared from the skin before 36 h, whereas DPU (log $P = 2.98$) begins to be eliminated approximately 20 h after treatment, as suggested by the onset of curvature in the permeation profile in Fig. 2.

A similar lipid disruption mechanism has been proposed for the action of several accelerants including Azone and oleic acid (Barry, 1987a; Barry, 1989; Goodman and Barry, 1989) Following a reduction in the barrier function of the stratum corneum, additional PG may enter the membrane, thereby further increasing partitioning of the urea analogues. DMI does not penetrate the stratum corneum well, does not promote partitioning of the test agents into the skin, and is thus a less effective vehicle for the administration of the penetration enhancers.

In conclusion, our data indicate that urea and the vehicles alone are ineffective in promoting permeation of 5-FU through human cadaver skin. The urea analogues are equally effective from a given vehicle, but are more effective when applied in PG compared with application from DMI or LLP. No correlation was found between the log partition coefficients (octanol/water) and enhancement ratios of the urea analogues, and the intervehicle variations in the enhancement ratios is not due to solubility differences. The results support the LPP theory for accelerant activity with

the analogues disrupting the lipid packing in the stratum corneum thereby increasing the membrane diffusivity to 5-FU.

Acknowledgements

The authors thank the Science and Engineering Research Council for a studentship for A.C.W., and Dr. J.V. Greenhill for assistance with the organic synthesis.

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