Pharmaceutical Research, Vol. 3, No. 5, 1986

Report

The Influence of Urea on Percutaneous Absorption

Janet Beastall,[1,2] Richard H. Guy,[1,2] Jonathan Hadgraft,[1-3] and Ian Wilding[1,2]

Received January 10, 1986; accepted March 10, 1986

The release characteristics of aqueous and oily creams (BP) containing urea up to concentrations of 10% have been studied. The results show that the urea has little influence on the ability of the formulation to release its active ingredient, in this case, hexyl nicotinate. The preparations were then assessed in an *in vivo* experiment by measuring the time of onset of erythema induced by the hexyl nicotinate. The urea influenced the time of onset of erythema, and a mechanism for its action is proposed.

KEY WORDS: penetration enhancers; percutaneous absorption; urea; hexyl nicotinate.

INTRODUCTION

The skin presents a very efficient barrier to topically applied drugs. In order to provide a high drug concentration to the lower regions of the skin, it is necessary to optimize the formulations that are used to deliver them. One way of achieving this is to maximize the thermodynamic activity of the drug by a suitable choice of solvents. Another method is to incorporate a penetration enhancer into the formulation. Many substances have been suggested as enhancers of percutaneous absorption, e.g., dimethyl sulphoxide, *N*-methyl pyrrolidone, dimethyl formamide, dimethyl acetamide, *N,N*-diethyl *m*-toluamide, urea, and 1-dodecylazacycloheptan-2-one (Azone). Although numerous candidates have been investigated, no conclusive evidence has been obtained concerning their mechanism of action.

The penetration of esters of nicotinic acid through the skin has been investigated extensively and the mechanism by which these esters transport documented. The route of penetration of the nicotinates is via the intercellular channels (1). According to Elias (2), these channels are lipid rich, and the lipids form a structured array. It is thus possible that penetration enhancers act by disrupting the structure of these lipids thereby increasing the permeability of the skin. In this publication we have investigated the penetration of hexyl nicotinate through the skin in the presence of urea in an attempt to probe its mechanism of action.

The nicotinates diffuse through the stratum corneum and reach the junction between the viable epidermis and the dermal vasculature at a sufficient concentration to create vasodilatation in a relatively short period of time (3). The time of onset of the induced erythema is one method of quantifying the permeability of the skin and also the release properties of the formulation which contains the nicotinate (4). If the formulations can be shown independently to release the nicotinate at the same rate, then any changes in the time of onset of erythema will be a result of the formulation having some effect on the barrier function of the skin.

THEORY

It has previously been shown that the transport of the nicotinates through the stratum corneum can be analyzed using solutions to Fick's second law of diffusion (5). The equation required to predict the relationship between the time of onset of erythema and the diffusion parameters of the skin is

$$\log\left(\frac{n_E}{\ell}\right) = \log(ct_E^{1.5}) + \log[K/(1 + Kp^{-1/2})] + \log[8\pi^{-1/2}(D/\ell^2)^{3/2}] - \ell^2/9.2Dt_E \quad (1)$$

where c is the concentration of the applied nicotinate, n_E is the concentration of nicotinate required to trigger erythema, t_E is the time of onset of erythema, K is the partition coefficient of the nicotinate between the formulation and the epidermis, p is the ratio of the diffusion coefficients of the nicotinate in the formulation and the skin, l is the diffusional path length of the nicotinate in the stratum corneum, and D is the diffusion coefficient of the nicotinate in the stratum corneum.

Thus there should be a linear relationship between log $(ct^{1.5})$ and $1/t_E$. The slope of the straight line obtained provides information about the diffusional characteristics of the skin and the intercept of the graph should show differences between the partitioning characteristics of the different formulations. If urea is added to a formulation and it is seen that there is an alteration in the gradient of the above graph, then the urea is affecting the diffusional characteristics of

[1] Department of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

[2] Departments of Pharmacy and Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143.

[3] To whom correspondence should be addressed at The Welsh School of Pharmacy, UWIST, P.O. Box 13, Cardiff CF1 3XF, U.K.

0724-8741/86/1000-0294$05.00/0

the skin. By observing the way in which the urea is acting as a function of the concentration, it should be possible to obtain some idea about the mechanism of action of this accelerant.

EXPERIMENTAL

For this study, simple aqueous and oily creams (BP) have been used with varying concentrations of urea, up to 10%. Urea was incorporated into the aqueous phase of the preparation and hexyl nicotinate (5%) into the lipid phase. The *in vitro* release studies were conducted in the simple cell shown in Fig. 1. The technique is an adaptation of that described by Billups and Patel (6). The membrane used to separate the base from the receptor phase (pH 7.4 phosphate buffer) was Visking tubing. The cells were thermostated at 30°C, and in no case was the membrane used in the experiment rate limiting. Thus the release profiles obtained were a true reflection of the ability of the base to liberate the active constituent, in this case, hexyl nicotinate. The concentration of the nicotinate in the receptor phase was measured by periodic sampling and assaying the solution by uv spectrophotometry at 263 nm.

Duplicate runs were performed and the results analyzed using Eq. (2):

$$M_t = 2AD^{1/2}t^{1/2}C_o\pi^{-1/2} \quad (2)$$

where M_t is the amount of drug in the receptor phase at time t, A is the membrane area, D is the apparent diffusion coefficient of the drug in the formulation, and C_o is the concentration of hexyl nicotinate in the formulation.

The results are shown in Fig. 2 and Table I. Figure 2 shows a typical graph, where it is seen that there is a good correlation between the amount of drug released and the square root of time. If the membranes in the diffusion cells were rate limiting, linear relationships would not have been observed. The results also show that urea has a small effect on the way in which the bases release the hexyl nicotinate *in vitro;* no correlation between the concentration of the urea and the alteration in the apparent diffusion coefficient could be found.

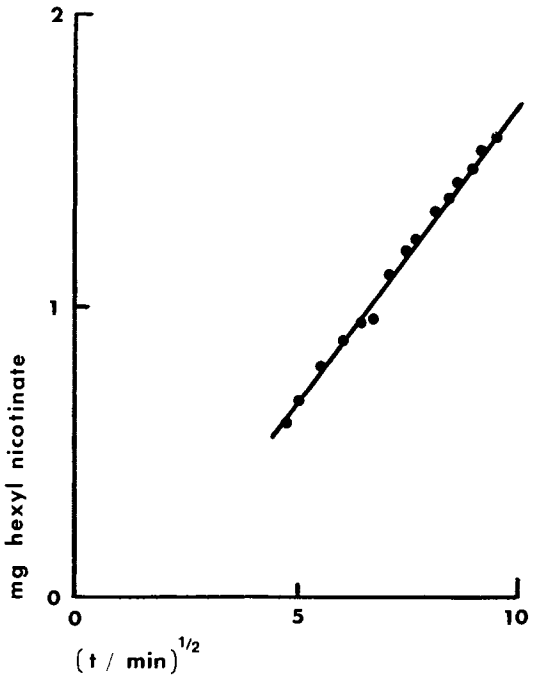

Fig. 2. Release of hexyl nicotinate (5%) from aqueous cream BP (0% urea) as a function of the square root of time.

The *in vivo* studies were conducted by applying a con-

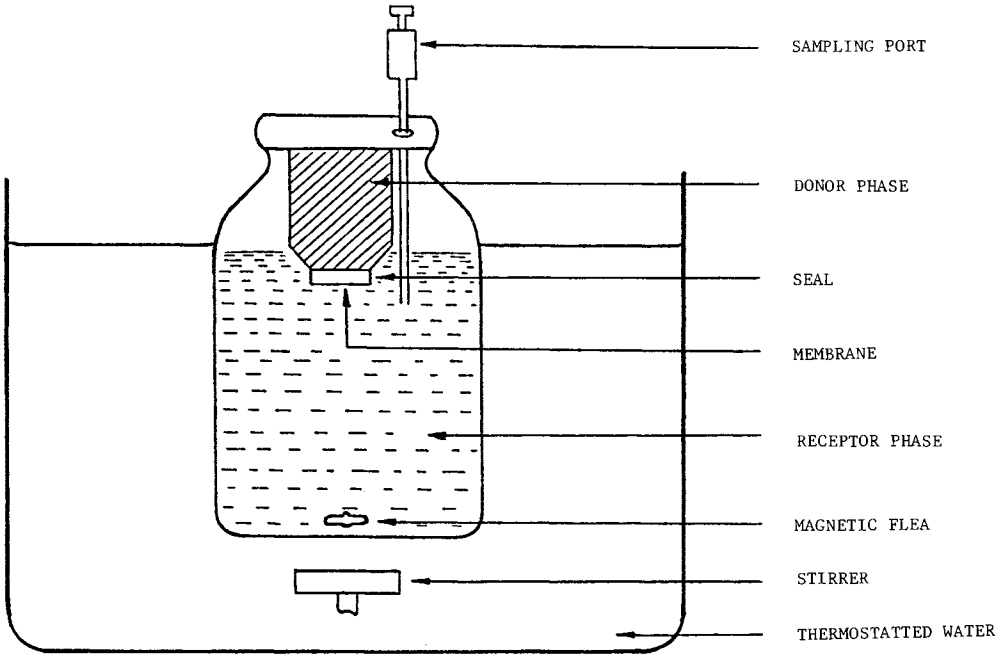

Fig. 1. Diagrammatic representation of the *in vitro* diffusion cell for assessing release from the formulations.

Table 1. Apparent Diffusion Coefficients for Varying Urea Formulations

% urea in formulation	Apparent diffusion coefficient ($cm^2\ sec^{-1}$) × 10^8
0	2.34
0.5	2.54
1	4.28
2	2.54
5	3.98
10	4.01

trolled amount of the formulation to the flexor aspect of the forearm as described by Barrett *et al.* (7). The time of onset of erythema was taken when a red halo was seen around the edge of the base. For the aqueous bases the time was measured as a function of the concentration of both the hexyl nicotinate and the incorporated urea. The results are presented in Table II. They are then subjected to the analysis described under Theory and plotted graphically (Fig. 3) according to Eq. (1). This shows that the results do comply with the theory, and the gradients and intercepts of the lines are given in Table III. In the case of the oily bases only the effect of urea concentration was studied; the results are given in Table IV.

RESULTS AND DISCUSSION

The release properties of the formulations are summarized in Table I. The apparent diffusion coefficient is influenced by the presence of urea but changes of this magnitude would not be expected to affect the *in vivo* performance of the preparation.

The *in vivo* results are presented in Table II. There is no statistical difference between the mean values quoted for formulations containing 0 and 5% urea; there is a difference between 0 and 10% and between 5 and 10% ($P = 0.05$). The times of onset of erythema follow the expected pattern, as the concentration of nicotinate increases, the times decrease. At higher urea concentrations the times are reduced. It is perhaps surprising that urea elicits its response as quickly as it does in reducing the barrier function of the stratum corneum. The *in vivo* data have been analyzed according to Eq. (1), and the results are given in Table III. The intercepts of the graphs in Fig. 3 are similar, which shows that the urea has had little effect on the partitioning behavior of the nicotinate between the base and the skin. If the urea was acting by changing the thermodynamic activity of the nicotinate, gross differences in the intercept would be predicted. The gradients of the lines show that there is a difference between 0 and 10% and between 5 and 10% but not between 0 and 5%. This result may be expected from a consideration of the statistical treatment in Table II. It is possible to calculate the apparent diffusion coefficient of the hexyl nicotinate in the skin if the diffusional length is estimated. Assuming that transfer is via the intercellular channels, we can estimate this length to be 340 μm (1,5) and a value for D can be calculated. The values are given in Table III. The results show that urea halves the resistance of the skin to hexyl nicotinate in a comparatively short period of time. The value obtained in the absence of urea is also comparable to that of methyl nicotinate ($D = 2.7 \times 10^{-11}\ m^2\ sec^{-1}$) obtained in previous experiments (1,5).

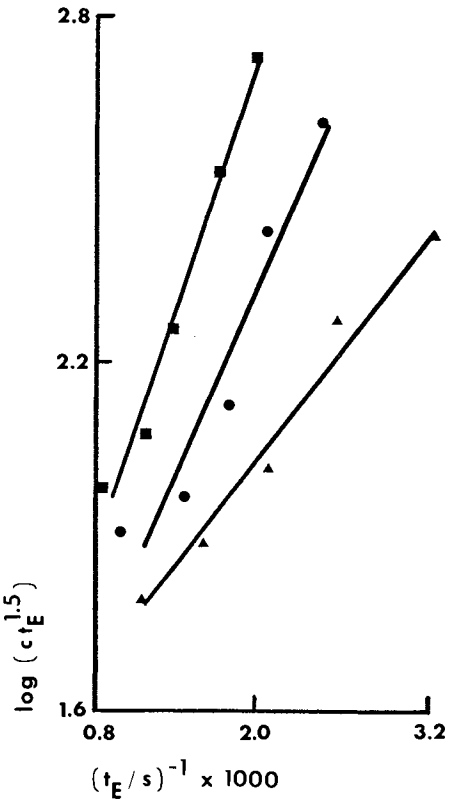

Fig. 3. *In vivo* data given in Table II plotted according to Eq. (1). (■) 0% urea; (●) 5% urea; (▲) 10% urea.

The data given in Table III suggest that urea acts rapidly on the structured lipids which comprise the intercellular channels. It does not alter the partitioning of the hexyl nicotinate from the base into the stratum corneum but it does

Table II. Time of Onset of Erythema [min (± SE)] for Hexyl Nicotinate in Aqueous Cream BP ($N = 20$)

Hexyl nicotinate (%)	Urea 0%	Urea 5%	Urea 10%
0.05	18.5 (0.48)	17.2 (0.44)	14.4 (0.22)
0.1	12.9 (0.49)	11.86 (0.28)	10.52 (0.15)
0.2	11.08 (0.35)	9.62 (0.34)	8.11 (0.15)
0.5	8.7 (0.43)	8.24 (0.39)	6.6 (0.16)
1	7.1 (0.3)	6.94 (0.28)	5.1 (0.26)

Table III. Gradients and Intercepts Calculated from Fig. 3 (The Corresponding Diffusion Coefficients Have Been Calculated Assuming an Intracellular Route)

Formulation	Gradient $l^2/9.2 \times 10^3\ D$ (± SE)	$D/m^2\ sec^{-1}$	Intercept
0% urea	0.62 (0.08)	2.02×10^{-11}	1.31
5% urea	0.53 (0.09)	2.4×10^{-11}	1.30
10% urea	0.32 (0.03)	3.9×10^{-11}	1.41

Table IV. Times of Onset of Erythema (N = 20) for Hexyl Nicotinate (1%) in Oily Cream BP Containing Increasing Concentrations of Urea

Concentration of urea (%)	Mean time for onset of erythema [min (±SE)]
0.0	11.6 (0.8)
0.5	10.4 (0.7)
1.0	8.6 (0.5)
2.0	8.6 (0.6)
5.0	6.7 (0.4)
10.0	6.5 (0.4)

affect the integrity of the lipids. The exact mechanism of this is unclear but it can be proposed that urea may lower the phase transition temperature of the lipids sufficiently that they are fluidized at the ambient temperature of the stratum corneum. Alternatively, it has been documented (8) that urea increases the hydration of skin. The increased water content in the lipids may swell their structure and render them more permeable. The results show that concentrations of greater than 10% urea are required for it to produce an effect, and this should be considered in any pharmaceutical formulation. It is interesting to note that the marketed product Calmurid HC (Pharmacia Ltd.) contains 10% urea (Hellgren and Larsson).

Urea also influences the time of onset of erythema if it is incorporated into oily bases. Table IV shows the times of onset of erythema for oily-base BP containing increasing concentrations of urea. The results also suggest the urea acts very quickly on the barrier function of the skin.

This analysis can be used to show the way in which penetration enhancers alter the barrier function of the skin and quantify the effect. It would be possible to investigate how long the barrier function is impaired by repeating the experiments with the nicotinates as marker compounds. With the current interest in transdermal drug delivery, this is obviously an area which may be exploited and one where further investigation is warranted.

ACKNOWLEDGMENTS

We thank Nelson Research and the NIH (GM-33395) for financial support.

REFERENCES

1. W. J. Albery and J. Hadgraft. *J. Pharm. Pharmacol.* **31**:140–147 (1979).
2. P. Elias. *J. Invest. Dermatol.* **80**:44–49s (1983).
3. C. W. Barrett, J. W. Hadgraft, and I. Sarkany. *J. Pharm. Pharmacol.* **16**(Suppl.):104T–107T (1964).
4. B. C. Lippold and A. Teubner. *Pharm. Ind.* **43**:1123–1133 (1981).
5. W. J. Albery and J. Hadgraft. *J. Pharm. Pharmacol.* **31**:129–139 (1979).
6. N. F. Billups and N. K. Patel. *Am. J. Pharm. Educ.* **34**:190–196 (1979).
7. C. W. Barrett, J. W. Hadgraft, and I. Sarkany. *Br. J. Dermatol.* **76**:479 (1964).
8. L. Hellgren and K. Larsson. *Dermatologica* **149**:289–293 (1979).