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Penetration enhancing effect of Azone on the transport of 5-fluorouracil across the hairless rat skin

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Summary

The effect of the penetration enhancer, Azone, on drug transport across skin was investigated using an in vitro permeation technique involving diffusion cells, hairless rat skin and 5-fluorouracil. The permeability of 5-fluorouracil across full-thickness skin (stratum corneum to dermis transport) was enhanced by Azone about 100-fold although a lag time of approximately 10 h was observed. This long lag time was not found after in vitro and in vivo pretreatments with Azone. Azone had no effect on the epidermis-to-dermis transport (across stripped skin) or dermis-to-stratum corneum transport (across reversed full-thickness skin). These results suggest that the amount of Azone in the skin, especially in the stratum corneum, may be related to its penetration enhancing effect. The results also suggest that incorporation of Azone into transdermal dosage forms could be useful in enhancing the transport of drugs for which the rate-limiting step for percutaneous absorption is penetration of the stratum corneum.

Introduction

The potential of skin as a site for administration of systemically active drugs has been recognized. Many penetration enhancers have, therefore, been examined in attempts to ensure that therapeutic efficacy may be attained. Dimethyl sulfoxide (DMSO) has been the most widely studied skin penetration enhancer, but because of its irritancy to the skin, its use in transdermal dosage forms is decreasing. Pyrrolidones, urea and propylene glycol also enhance drug transport across skin (Barry, 1983), but generally the extent of enhancement is less than that of DMSO. Rajadhyaksha (1976), Stoughton (1982a and b) and Stoughton and McClure (1983) have suggested the use of 1-dodecylazacycloheptan-2-one (Azone) (Fig. 1) in topical combinations, especially for antibacterials, antifungals and steroids. Azone is a colorless, odorless and pharmaceutically inert liquid which has a smooth, oily, nongreasy feel. It is insoluble in water, but is freely soluble in most organic solvents, including the lower organic alkanols. It is stable for at least 7 years when stored at room temperature. Other properties can be obtained from the Azone Product Profile (Nelson Research and Development, 1984). In previous work (Sugibayashi et al., 1985),

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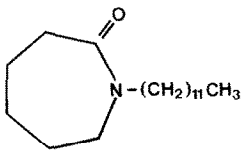

Fig. 1. Chemical structure of Azone.

we have suggested that Azone enhances drug transport across full-thickness skin. Azone can be applied directly to denuded skin with no significant discomfort even at concentrations above 50% (Stoughton, 1982a). Its mechanism of action is unknown.

In the present study, we have used 5-fluorouracil (5-FU) as a model drug to attain a better understanding of the mode of the transdermal absorption enhancing action of Azone. All experiments were conducted at low (tracer) levels of 5-FU using 2-chamber diffusion cells.

Materials and Methods

Materials

5-[6-^{3}H]Fluorouracil (spec. act. 7.68 mCi/mg; greater than 99% purity) was purchased from Amersham Japan (Tokyo) and used after the radiochemical purity was checked by TLC (TLC plates, Merck Silica Gel F254; solvent, ethyl acetate:methanol (75 : 25)). Azone was kindly supplied by Nelson Research and Development (Irvine, CA, U.S.A.). Polysorbate 20, Triton X-100 and poly(acrylic acid) (Carbopol 934) were purchased from Wako Pure Chemical Industries (Osaka, Japan), New England Nuclear (Boston, MA, U.S.A.) and B.F. Goodrich Chemical Co. (Cleveland, OH, U.S.A.), respectively. All other chemicals were reagent grade and obtained commercially.

Animals

Male hairless rats (WBN/kob strain) (Sugibayashi and Morimoto, 1984), with an approximate weight of 150 g each, were supplied by Saitama Laboratory Animals (Sugito, Saitama, Japan). Wistar rats were also used so that a comparison of the permeability of 5-FU in these and in hairless rats could be made.

Diffusion cell

A diffusion cell consisting of two half cells, reported by Yu et al. (1979), was further modified by a local glass shop. Each half cell has a volume of 2 ml and an effective diffusional area of 0.636 cm^2. A 15-cm stirring shaft was attached to each half cell. The stirrer, made of polytef and equipped with a teflon propeller ($\sim$ 8 mm in length), was driven by a 150 rpm constant speed motor. The sampling port, with a stopper for each half cell, was 1 cm in length and 3 mm in diameter.

Skin membrane preparation

The abdominal region of the hairless and Wistar rats was carefully shaved. A 2 cm^2 section of the left and right abdominal skin was mounted between two half cells and clamped immediately after the skin section was excised from each anesthetized rat (sodium pentobarbital, 60 mg/kg i.p.). Stripped skin was obtained by stripping the stratum corneum from the shaved abdominal skin with adhesive cellophane tape (24 mm width, Nichiban Co., Tokyo) (Washitake et al., 1973).

Permeation procedure

The dermis side of the skin was in contact with the receiver compartment and the stratum corneum (full-thickness skin) or the epidermis (stripped skin) with the donor compartment. To check the effects of Azone on the dermis, 5-FU permeation from the dermis side to the stratum corneum was measured using the reversed system. The receiver compartment of each cell was filled with 2 ml of saline (0.9% NaCl) and the donor compartment with 2 ml of o/w emulsion consisting of 3.3% (w/v) Azone and 0.11% (w/v) polysorbate 20 (as an emulsifier) in saline (Azone treatment) or with 2 ml of saline alone (control). After the diffusion cell had been allowed to attain equilibration at a temperature of 37°C in a water bath (usually 5 min), an aliquot (0.2 ml) was withdrawn from the donor side and 0.2 ml of an aqueous solution, containing 4 μCi of [^{3}H]5-FU, was added. The concentrations of Azone and polysorbate 20 were then 3 and 0.1% (w/v), respectively, at the beginning of the permeation experiment. The donor and receiver compartments were stirred continuously at 150 rpm. At appropriate times, 10 and

100 μl samples were withdrawn from the donor and receiver compartments, respectively, and transferred to a vial containing 7 ml of scintillation cocktail (composition: 4 g PPO, 0.4 g POPOP, 1.0 liter toluene, 0.5 liter Triton X-100). Radioactivites were assayed on an Aloka scintillation counter (LSC 700, Tokyo). After sampling, 100 μl of saline were added to the receiver compartment to keep the volume constant but the decrease in donor compartment volume was ignored.

Pretreatment with Azone

The effect of in vitro pretreatment with Azone or saline on the permeability of 5-FU was investigated using the same method except that the skin was pre-soaked with Azone or saline between two half diffusion cells (one cell facing the stratum corneum contained 3% (w/v) of Azone and 0.1% (w/v) polysorbate 20 in saline or saline and the other contained saline alone) for 8 or 16 h. In vivo Azone pretreatment was as follows. 2 g of 3% Azone aqueous gel ointment (0.8% Carbopol 934, 2% 2.5 N NaOH) with a backing of Catheripad (Nichiban Co.) were applied on a 22 cm^2 area of abdominal skin for 24 h. Gel ointment without Azone was also used for comparison purposes. In vitro permeation experiments were the same as above.

Calculation method

Data are presented as Q(%) (percentage of cumulative amount of penetrant permeated per unit area against the initial loading amount, %/cm^2) and P (permeability coefficient, cm/s) as follows.

$$Q(\%) = \frac{C_t \cdot V_r}{C_{in} \cdot V_d} / A \qquad (1)$$

$$P = (dC_t/dt) \cdot V_r/A/\Delta C \qquad (2)$$

where C_t, C_{in}, V_r, V_d and A are the penetrant concentration in the receiver solution, initial concentration of penetrant in the donor solution, volume of receiver or donor solution, and diffusion area, respectively; and dC_t/dt and ΔC are the change in penetrant concentration in the receiver cell vs time and the concentration differential across skin. The time course of P was determined by calculating the slope (dC_t/dt) at each midpoint between two sampling intervals and correcting for the decrease in ΔC using the penetrant concentration in the donor and receiver solutions at each sampling time. Data are presented as the mean or mean ± S.E.M. of 3–5 experiments and statistical analyses were performed using Fisher's *t*-test.

Results

Comparison of in vitro permeation through hairless and Wistar rat skins

Since WBN/kob rats have not previously been used for percutaneous absorption experiments, the time course for 5-FU permeation across hairless rat skin was determined and compared with that across Wistar rat skin. Fig. 2a and b show the time courses of the percentage of cumulative amount of 5-FU permeated, Q(%), across the full-thickness and stripped skin, respectively. The amount of 5-FU which permeated across the hairless rat skin was not significantly different from that across the Wistar rat skin ($P > 0.05$ for both tissues).

The Q(%) values across the stripped hairless rat skin was about 30% smaller than that across Wistar rat skin, but both sets of data were not significantly different ($P > 0.05$).

Although there are small differences between the transport profiles in hairless and Wistar rats, the time course of 5-FU transport across the skin of both strains was similar as shown in Fig. 2. Since there was no significant difference in 5-FU transport across the 2 types of skin, only hairless rats were used for subsequent experiments.

Many drugs have been found to be degraded and metabolized in viable skin (Hadgraft 1980). Thin-layer chromatograms of 5-FU permeated through skin showed a single peak owing to 5-FU. This suggests that it is very stable to enzymatic degradation in the skin of both strains of rats (data not shown).

Effect of Azone on the transport of 5-FU across the full-thickness skin

The effect of Azone on the transport of 5-FU

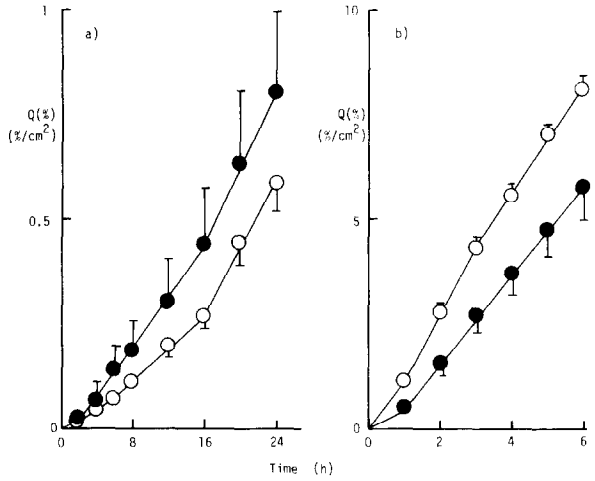

Fig. 2. Comparison of 5-FU transport across full-thickness skin (a) and stripped skin (b) in hairless rats with that of Wistar rats. Hairless rat (●); Wistar rat (○).

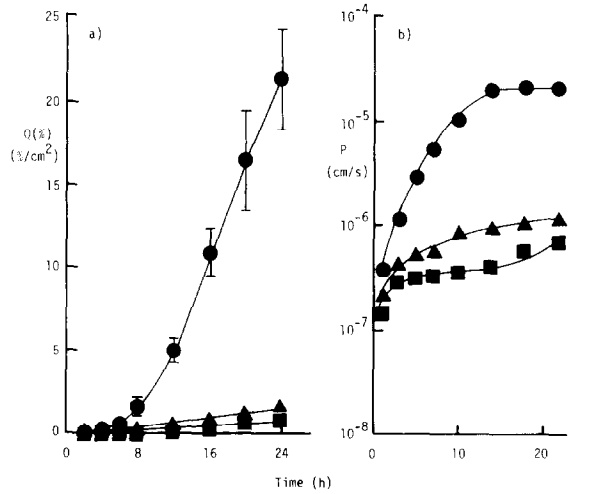

Fig. 3. Effect of Azone on 5-FU transport across full-thickness skin. (a) Time course of Q(%) with Azone (●), polysorbate 20 (▲), and saline alone (■). (b) Time course of P; symbols as in (a).

across full-thickness skin was investigated. Fig. 3a shows the time courses of Q(%) for full-thickness skin in a system containing 3% (w/v) Azone (containing 0.1% (w/v) polysorbate 20 for emulsification), 0.1% (w/v) polysorbate 20 alone and saline alone in the donor cell. Azone enhanced the drug transport markedly (10–100 times) although there was a lag time of approximately 8–10 h. The lag time was estimated by extrapolation of the Q(%) versus time plot at steady state to Q(%) zero. Q(%) values (0.0170 ± 0.0058 %/cm^2 at 8 h and 0.2112 ± 0.0306 %/cm^2 at 24 h) were significantly different from those of saline treatment ($P < 0.02$ at 8 h; $P < 0.001$ at 24 h; refer to Fig. 2a for the exact data for saline treatment) and polysorbate treatment (significant differences are the same as for saline treatment).

The time course of log P is shown in Fig. 3b, which shows the increase in penetration of 5-FU across skin with time more clearly than Fig. 3a. Increases in the permeability coefficient for saline treatment may be due to skin hydration (Behl and Barret, 1981). Throughout the experiment, the permeability coefficient of 5-FU in the presence of polysorbate 20 was about double that in the presence of saline. In the presence of Azone, the permeability coefficient of 5-FU increased markedly with time up to 14 h and at steady-state it was much higher (2.0×10^{-5} cm/s) than that obtained with either saline or polysorbate treatment.

Effect of Azone pretreatment on the transport of 5-FU

To attempt to explain the long lag time shown in Fig. 3, Azone pretreatment was carried out on full-thickness skin. Fig. 4a shows the time course of Q(%) after pretreatment with Azone or with saline without 5-FU for 8 or 16 h. The long lag time seen in the non-pretreatment experiment disappeared with Azone pretreatment (Azone–Azone systems) (the lag times with Azone–Azone system were 15 and 40 min for 16 and 8 h pretreatment, respectively). The transport profile with saline after pretreatment with saline for 16 h (saline–saline system) was similar to the control (saline) experiment in Fig. 3. However, the flux obtained with saline after pretreatment with Azone (Azone–saline system) was not significantly different ($P > 0.05$) from that of the Azone–Azone system although it was a little smaller.

Fig. 4b shows the permeability profiles of these systems. The permeability coefficient of the Azone–Azone system did not change with time after the lag time and the lag time period may be explained by the initial diffusion of 5-FU into the

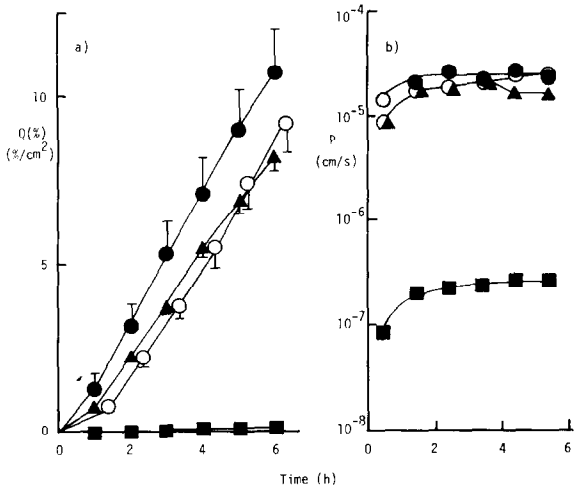

Fig. 4. Effect of in vitro Azone pretreatment on 5-FU transport across full-thickness skin. (a) Time course of Q(%) for Azone (16 h)–Azone system (●), Azone (8 h)–Azone system (○), Azone–saline system (▲) and saline–saline system (■). (b) Time course of P; symbols as in (a).

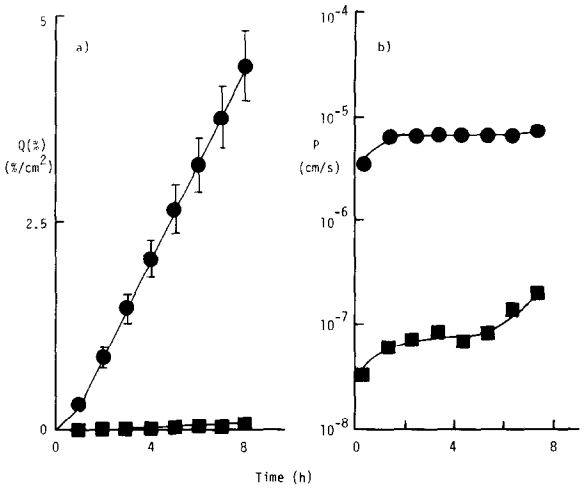

Fig. 5. Effect of in vivo Azone pretreatment on 5-FU transport across full-thickness skin. (a) Time course of Q(%) with (●) and without Azone (■). (b) Time course of P; symbols as in (a).

skin. The average permeability coefficients are almost the same as the level at steady-state for the Azone system without pretreatment (Fig. 3), suggesting that Azone might have a maximum effect. The flux with the Azone–saline pretreatment system was retained even though only saline was present during the 5-FU permeation experiment; the permeation rate of 5-FU decreased to about 80–90%. This result and the long lag time seen in the Azone treatment without Azone pretreatment suggest that the existence of Azone in the skin might be a key factor in enhancing the transport of 5-FU across skin.

Pretreatment with Azone in the diffusion cells may create a problem with regards to skin hydration and a long in vitro soaking period may simulate an in vivo situation. To show if the in vitro penetration mechanism modelled the clinical mechanism, Azone treatment was carried out in vivo. Poly(acrylic acid) gel ointment with a Catheripad backing was used for in vivo Azone pretreatment (the Azone concentration was adjusted to 3% as in the polysorbate 20 emulsions). Fig. 5a and b show Q(%) and P with Azone (in vivo pretreatment with Azone ointment and in vitro treatment with Azone emulsion) and without Azone (in vivo pretreatment with ointment base and in vitro treatment with saline). The permeability increased initially and about 20 h after starting the saline treatment. The first and second increases might be due to the initial diffusion of penetrant and skin hydration, respectively. The permeability coefficient was a little smaller than that seen in saline treatment in vitro as shown in Fig. 3b. In contrast, a marked enhancement in the transport of 5-FU was found after Azone ointment pretreatment. A large steady-state flux without a large lag time was observed. The permeability coefficient after in vivo Azone ointment pretreatment, however, was about one-third that obtained with the in vitro Azone emulsion pretreatment in the diffusion cell. The difference may be explained by skin hydration during in vitro pretreatment.

Effect of Azone on the epidermis and dermis

From the results, it is clear that Azone enhances 5-FU transport markedly. One of the major questions which arises from these results is Azone's site of action. In an attempt to clarify this, the effects of Azone on the transport of 5-FU through the epidermis (without the stratum corneum) and dermis were measured.

Fig. 6a and b show Q(%) and P values across stripped skin, which lacks the stratum corneum,

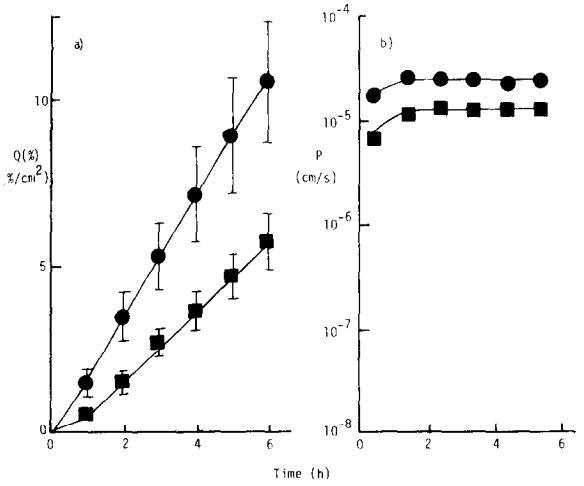

Fig. 6. Effect of Azone on 5-FU transport across the stripped skin. (a) Time course of Q(%) with (●) and without Azone (■). (b) Time course of P; symbols as in (a).

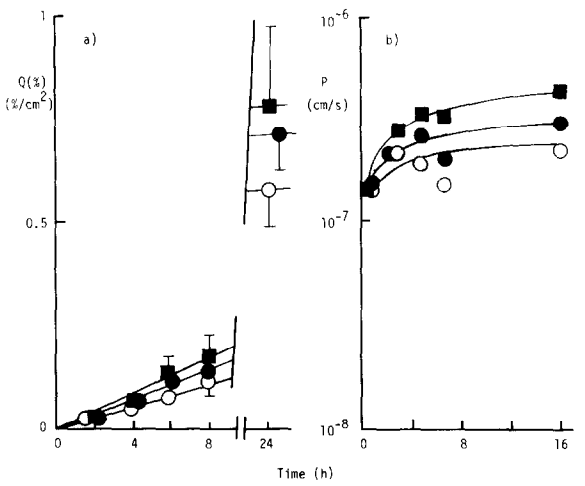

Fig. 7. Effect of Azone on 5-FU transport from the dermis to the stratum corneum. (a) Time course of Q(%) with (●) and without Azone (○) from the dermis to the stratum corneum and without Azone from the stratum corneum to the dermis (■). (b) Time course of P; symbols as in (a).

TABLE 1

SUMMARY OF Q(%) VALUES AT THE FINAL SAMPLING POINT AND P VALUES AT STEADY-STATE

Experimental system	Q(%) (%/cm^2) [a]	P (cm/s) [b]	Cited figure
hairless rat			
full-thickness skin	0.0080 ± 0.0021 (24 h)	–	2
Wistar rat			
full-thickness skin	0.0058 ± 0.0007 (24 h)	–	
hairless rat			
stripped skin	0.0571 ± 0.0081 (6 h)	–	
Wistar rat			
stripped skin	0.0815 ± 0.0011 (6 h)	–	
saline	0.0080 ± 0.0021 (24 h)	$\approx 4 \times 10^{-7}$	3
polysorbate 20	0.0168 ± 0.0013 (24 h)	$\approx 8 \times 10^{-7}$	
Azone	0.2112 ± 0.0306 (24 h)	$\approx 2 \times 10^{-5}$	
saline-saline	0.0010 ± 0.0004 (6 h)	3.6×10^{-6}	4
Azone (8h)-Azone	0.0926 ± 0.0096 (6 h)	2.1×10^{-5}	
Azone (16h)-Azone	0.1071 ± 0.0125 (6 h)	2.4×10^{-5}	
Azone-saline	0.0823 ± 0.0051 (6 h)	1.8×10^{-5}	
saline (in vivo)-saline	0.0007 ± 0.0002 (8 h)	1.0×10^{-7}	5
Azone (in vivo)-Azone	0.0446 ± 0.0042 (8 h)	6.7×10^{-6}	
saline (stripped skin)	0.0572 ± 0.0081 (6 h)	1.2×10^{-5}	6
Azone (stripped skin)	0.1046 ± 0.0186 (6 h)	2.2×10^{-5}	
saline			
(to stratum corneum)	0.0055 ± 0.0001 (24 h)	$\approx 2 \times 10^{-7}$	7
Azone			
(to stratum corneum)	0.0070 ± 0.0002 (24 h)	$\approx 3 \times 10^{-7}$	
saline (to dermis)	0.0080 ± 0.0021 (24 h)	$\approx 3 \times 10^{-7}$	

[a] Percentage of cumulative amount of 5-FU at the end of experiment; average ± S.E.M.

[b] Permeability coefficient at steady-state.

with and without Azone. The values of Q(%) and P obtained with Azone treatment were greater than the values obtained without Azone but there was no significant difference between both Q(%) and P for both treatments. The small lag times were probably due to the initial diffusion of 5-FU into the skin so a steady state could be attained. These results show that Azone has little effect on stripped skin but has marked effects on the stratum corneum, which is the biggest barrier to percutaneous absorption for most drugs.

The permeability coefficient for stripped skin with Azone (Fig. 6b) was essentially the same as that obtained for full-thickness skin at steady-state (Fig. 3b) and that with in vitro pretreatment (Fig. 4b). All of these permeability coefficients were about 2×10^{-5} cm/s and we suggest that the permeability across stripped skin with Azone might be the maximum value.

To check the effect of Azone on the dermis, Azone emulsions were administered to it in the diffusion cells. In this case, the drug diffuses from the dermis through the epidermis to the stratum corneum. Fig. 7 shows Q(%) values for the permeation of 5-FU from the dermis to the stratum corneum with and without Azone. Data for the permeation from stratum corneum to dermis without Azone are added for comparison. It is clear that Azone does not have penetration enhancing effects in the dermis because there is no significant difference ($P > 0.05$) between the results obtained with and without Azone. These flux values were also very close to those obtained from stratum corneum to dermis without Azone.

Q(%) values at the final sampling time and P values at steady-state period for all experiments are summarized in Table 1.

Discussion

Since there is no significant difference in the transport profiles of 5-FU across skin between normal rats and hairless rats, the hairless rats might be useful for experiments using the skin if some problems such as breeding could be overcome.

Azone enhanced the 5-FU permeability across the full-thickness skin about 100 times. However, a long lag time (8–10 h) was seen which was probably due to the slow penetration of Azone into the skin. Pre-treatment with Azone was useful in decreasing the lag time to one which was due only to the initial diffusion of penetrant through the skin. In the case of multiple drug applications on the same site of skin, the low flux during the lag time may be ignored after the second administration. The small effect of Azone on both 5-FU permeations from the epidermis (to the dermis across stripped skin) and from the dermis (to the stratum corneum across full-thickness skin) suggests that the enhancer mainly affects the stratum corneum. Azone seems to change the diffusivity of 5-FU in the stratum corneum but is not so effective in terms of the diffusivities in the epidermis and dermis. The diffusivity in the stratum corneum increases to a steady state level after 14 h. This might explain the long lag time. It is suggested from the results of the 5-FU transport experiments from the dermis to the stratum corneum with Azone treatment, that permeation of Azone itself across the dermis might be very slow.

Azone enhanced the permeability of viderabine and its prodrugs across hairless mouse skin, 100–1000 times (Shannon et al., 1985), whereas it enhanced the permeability of steroids such as triamcinolone acetonide 2–5 times (Stoughton, 1982b). It is clear that Azone would be very useful for enhancing the permeability of hydrophilic compounds such as 5-FU and viderabine, because the rate-limiting layer for the percutaneous absorption of such drugs is the stratum corneum. In contrast, Azone might not be so effective for enhancing the permeations of liphophilic compounds because of their intrinsic permeability across the stratum corneum (Flynn et al., 1981). If Azone acts only on the stratum corneum, which contains keratin or sulfur-rich proteins, its low effect on the dermis and epidermis could be explained and its low effect on absorption from the gastrointestinal tract, rectum, vagina and other mucous membranes might be predicted. Further experiments are needed to interpret fully the mechanisms of action of Azone.

In vitro and in vivo experiments with high (saturation) levels of 5-FU are presently under

way in our laboratory for examining suitable concentrations of Azone using several hydrogel bases. We are also measuring Azone concentration in skin and Azone effect on the hydration of skin in order to clarify the mechanism and mode of action of Azone.

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