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# Effect of the absorption enhancer, Azone, on the transport of 5-fluorouracil across hairless rat skin

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The effect of the percutaneous absorption enhancer, Azone, on the transport of 5-fluorouracil across hairless rat skin has been investigated by an in-vitro permeation technique using 2-chamber diffusion cells. Azone (3% w/v) emulsions were used. Azone enhanced the permeability of drug 10–100 times across the full-thickness skin although there was a lag time about 10 h. The long lag time, however, disappeared with Azone pretreatment. Azone also affected the transport across stripped skin. These results suggest that Azone mainly affects the stratum corneum. It seems to change the diffusivity of drug in that layer and is not so effective against diffusivities in the epidermis and dermis.

Azone (1-dodecylazacycloheptan-2-one) has been reported as a new skin penetration enhancer for a number of drugs (Stoughton 1982a; Stoughton & McClure 1983). Although the mechanism of action is unknown, Azone can be applied directly to denuded skin with no significant discomfort even at concentrations above 50% (Stoughton 1982b). In the present study, we used 5-fluorouracil (5-FU) as a model drug in order to understand the mechanism of the penetration enhancing effects of Azone. All experiments were conducted at low (tracer) levels of 5-FU.

## *Materials and methods*

5-[6-$^3$H]FU (specific activity 7·68 mCi mg$^{-1}$, purity >99%) was purchased from Amersham Japan, and Azone was kindly supplied by Nelson Research and Development (Irvine, CA, USA). For the skin membrane permeation experiments, each section of left and right abdominal skin of WBN/kob hairless rats (Saitama Laboratory Animals, Japan), ca 150 g, was excised and mounted between two half diffusion cells, each having 2·0 ml volume and 0·636 cm$^2$ effective diffusional area (Yu et al 1979). One section of the skin was used for the permeation experiment with Azone and the other without Azone. Stripped skin (skin tape-stripped 20 times to remove the stratum corneum, Washitake et al 1973), was also used for comparison. The receiving compartment (dermis side of the skin) of each half cell was filled with 2 ml of 0·9% NaCl (saline) and the donor compartment (stratum corneum side for the full-thickness skin or epidermis for the stripped skin) was filled with 2 ml of 3·3% w/v Azone and 0·11% w/v polysorbate 20 in saline, 0·11% w/v polysorbate 20 in saline or saline alone. Polysorbate 20 was used as an emulsifier and the resulting mixture of Azone, polysorbate 20 and saline was an o/w emulsion. The diffusion cells were maintained at 37 °C in a water bath.

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An aliquot (0·2 ml) of solution was withdrawn from the donor side and 4 μCi of [$^3$H]5-FU was added as 0·2 ml of an aqueous solution. The concentrations of Azone and polysorbate 20 were 3 and 0·1% w/v, respectively at the beginning of the permeation experiments. The donor and receiver compartments were mixed throughout the experiment with a Teflon stirrer driven by a 150 rev min$^{-1}$ constant speed motor. 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 scintillation cocktail (composition; 4 g PPO, 0·4 g POPOP, 1 litre toluene, 0·5 litre Triton X-100). Radioactivities 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 volume was ignored. The effect of pretreatment with Azone on the permeability of 5-FU was checked with the same methods except that the skin was pre-soaked with Azone between two half diffusion cells for 8 h. The cell facing the stratum corneum contained 3% w/v of Azone and 0·1% w/v polysorbate 20 in saline and the other cell contained saline.

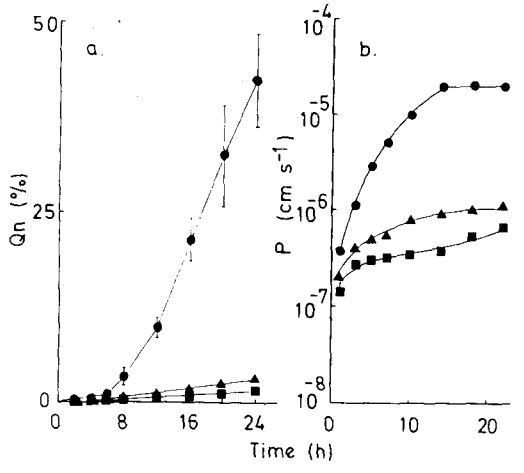


FIG. 1. Effect of Azone on the transport of 5-FU across the full-thickness skin of hairless rats. (a) Time course of $Q_n$ with Azone (●), polysorbate 20 (▲) and saline alone (■). (b) Time course of P; symbols as in (a).

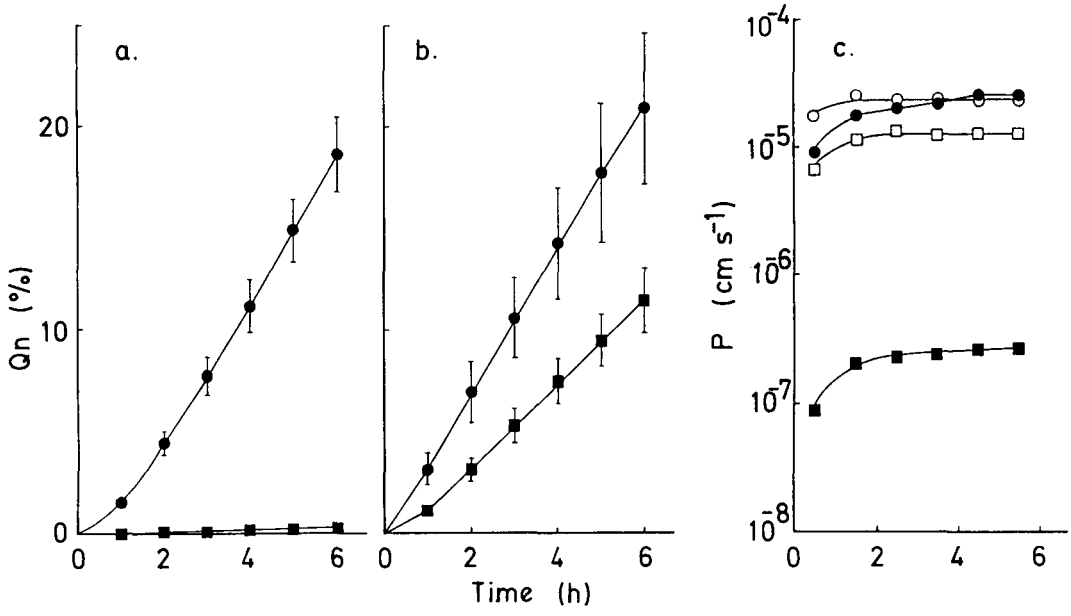


FIG. 2. Effect of Azone pretreatment and Azone treatment on the transport of 5-FU across the full-thickness skin and the stripped skin of hairless rats. (a) Time course of $Q_n$ across the full-thickness skin with (●) and without (■) Azone pretreatment. (b) Time course of $Q_n$ across the stripped skin with (●) and without (■) Azone treatment. (c) Time course of P across the full-thickness skin with (●) and without (■) Azone pretreatment and across the stripped skin with (○) and without (□) Azone treatment.

The cumulative amount of 5-FU per unit area permeated across the membrane, Q, is shown using the following equation.

$$Q = \frac{Ct\,V}{A} \quad (1)$$

where Ct, V and A are the penetrant concentration in the receiver solution, volume for receiver solution and diffusion area, respectively. To normalize the Q-values for initial concentration differences in the donor compartment ($C_{in}$), a term $Q_n$, was defined.

$$\%Q_n = Q/C_{in} \times 100 \quad (2)$$

The permeability coefficient, P, is estimated using the following equation.

$$P = \frac{dCt/dt\,V}{A\,\Delta C} \quad (3)$$

where dCt/dt and $\Delta C$ are the slope for the penetrant concentration in the receiver cell vs time and the concentration differential across the skin. Data are presented as $Q_n$ and P.

*Results*

Fig. 1a, b show the time course of the cumulative amount ($Q_n$) and permeability (P) of 5-FU across the full-thickness skin. Thin layer chromatograms of these samples showed a single peak due to 5-FU, which suggests that 5-FU is stable against enzymes in the skin (data not shown). The amount of drug penetrated over 24 h was 1·67 ± 0·42 (s.e.) % for saline (control) and 3·37 ± 0·27% for the polysorbate 20 treatment. The effect of polysorbate 20 was to double the permeability coefficient (i.e. about $6 \times 10^{-7}$ cm s$^{-1}$ for polysorbate, $3 \times 10^{-7}$ cm s$^{-1}$ for saline). In contrast, Azone enhanced the permeability markedly (10–100 times) although there was a lag time of about 10 h. $Q_n$ at 24 h was 42·24 ± 6·12 and the P value at steady state was $2 \times 10^{-5}$ cm s$^{-1}$.

Fig. 2a shows the time course of $Q_n$ after pretreatments with Azone or with saline without 5-FU for 8 h. The long lag time seen in the non-pretreatment experiment (see Fig. 1) disappeared with Azone pretreatment (it was less than 1 h). The permeation profile after pretreatment with saline was the same in the control (saline) experiment in Fig. 1.

Fig. 2b shows the effect of Azone on $Q_n$ using stripped skin. $Q_n$ values obtained with Azone were double those obtained without it. Lag time was about 10 min, which is probably due to the initial diffusion of 5-FU in the skin until the steady state.

The permeability coefficients for full-thickness skin after pretreatment with Azone (Fig. 2a) and for stripped skin (Fig 2b) are summarized in Fig. 2c. The permeability coefficient for the full-thickness skin after pretreatment with Azone was similar to that for stripped skin without pretreatment. In addition, the permeability coefficient for the full-thickness skin with Azone at steady state (non-pretreatment, Fig. 1) was also similar.

*Discussion*

The maximum effect of Azone on the permeability of 5-FU across the hairless rat skin was to increase it about 100-fold. With multiple drug applications, however, low fluxes during the lag time may be ignored after the second administration. The small effect of Azone on the permeability across the stripped skin suggests that Azone mainly affects the stratum corneum. Azone seems to change the diffusivity of 5-FU in the stratum corneum and is not so effective against the diffusivities in the epidermis and dermis. Therefore, Azone would be useful for enhancing the permeability of hydrophilic compounds such as 5-FU, because the rate limiting layer for the percutaneous absorption of such drugs is the stratum corneum. In contrast, Azone might not be effective for enhancing the permeability of lipophilic compounds due to their intrinsic large permeability of the stratum corneum (Flynn et al 1981).

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# Location of the mechanism of the clonidine withdrawal tachycardia in rats

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Withdrawal of chronic infusion of clonidine elicits severe tachycardia and short-lasting blood pressure elevations (upswings). Withdrawal of clonidine in low dosage (30 μg kg$^{-1}$ day$^{-1}$ i.c.v., 7 days) elicited a maximum of 10·9 ± 0·5 upswings h$^{-1}$. Cessation of s.c. infusion of clonidine (30 μg kg$^{-1}$ day$^{-1}$ 7 days) evoked a maximum of 1·9 ± 0·5 upswings h$^{-1}$. After cessation of the two clonidine infusions no overshoot of heart rate occurred. Withdrawal of a higher dose of clonidine (300 μg kg$^{-1}$ day$^{-1}$ s.c., 7 days), however, induced tachycardia (from 302 ± 8 to 433 ± 8 beats min$^{-1}$) and 7·6 ± 1·4 upswings h$^{-1}$. The administration of the $\alpha_2$-adrenoceptor antagonist yohimbine precipitated withdrawal tachycardia in animals treated with oxymetazoline, a hydrophilic α-adrenoceptor agonist. Yohimbine (3 mg kg$^{-1}$ i.p.) precipitated a severe rise in heart rate from 285 ± 14 to 520 ± 5 beats min$^{-1}$ in oxymetazoline (300 μg kg$^{-1}$ day$^{-1}$ s.c., 7 days) treated rats and from 320 ± 13 to 420 ± 11 beats min$^{-1}$ in saline-treated animals. Upswings were not induced by yohimbine treatment. It is concluded, that the blood pressure upswings after clonidine withdrawal are due to a central mechanism, whereas the mechanism of the overshoot of heart rate is located peripherally, probably at the cardiac presynaptic level.

Discontinuation of chronic treatment with clonidine (500 μg kg$^{-1}$ day$^{-1}$ s.c., 12 days) in normotensive rats elicits severe tachycardia (from 313 ± 4 to 456 ± 5 beats min$^{-1}$) and short-lasting intermittent blood pressure elevations (upswings) (8·5 ± 0·9 h$^{-1}$) (Thoolen et al 1981). The blood pressure upswings appear to be of central origin, since i.c.v. injection of the hydrophilic imidazolidine derivative oxymetazoline (30 μg kg$^{-1}$) abolishes the upswings after clonidine withdrawal (500 μg kg$^{-1}$ day$^{-1}$ s.c., 12 days), whereas i.p. administered oxymetazoline (30 μg kg$^{-1}$) does not affect the frequency of the upswings (Thoolen et al 1983). An additional argument in favour of a central mechanism is the suppression of the upswings by i.c.v. injection of 0·3–10 μg kg$^{-1}$ morphine, whereas s.c. administered morphine supresses the upswings at 1–3 mg kg$^{-1}$ (Thoolen et al 1983).

The origin of the withdrawal tachycardia is less clear. Morphine (0·3–10 μg kg$^{-1}$ i.c.v. or 1–3 mg kg$^{-1}$ s.c.) does not suppress the withdrawal tachycardia, but oxymetazoline (30 μg kg$^{-1}$ day$^{-1}$ i.c.v. or i.p.) abolishes this effect (Thoolen et al 1983). This finding points towards a possible involvement of a peripherally located mechanism in the development of the withdrawal tachycardia.

To elucidate the locations of the withdrawal mechanisms, the effects on blood pressure and heart rate of discontinuation of i.c.v. and s.c. infusions of clonidine were studied. In contrast to clonidine, the α-adrenoceptor agonist oxymetazoline does not cause withdrawal symptoms (as such) upon cessation of treatment, due to its long half life. For this reason withdrawal phenomena were provoked by means of $\alpha_2$-adrenoceptor blockade with yohimbine.

*Materials and methods*

Male, normotensive Wistar rats (350–400 g, Cpb/Wv-strain, TNO, Zeist, The Netherlands) were used. Permanently indwelling catheters were implanted under hexobarbitone sodium anaesthesia (150 mg kg$^{-1}$ i.p.) in

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