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Duration and Reversibility of the Penetration-Enhancing Effect of Azone

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In order to establish the duration and reversibility of the action of Azone, the permeation of 5-fluorouracil (5-FU) across the excised skin at 0, 1, 3 and 7 d after a 24 h pretreatment with Azone gel in hairless rats was measured. The longer the time after the pretreatment, the less Azone remained in the rat skin. The skin permeation of 5-FU was also decreased with the passage of time after the pretreatment. The amont of Azone at 7 d after the pretreatment was negligible in the skin compared to that at 0 d after the pretreatment, and the permeation rate of 5-FU across the skin at 7 d after the pretreatment was almost the same as that of the control (without Azone pretreatment). A good relationship between the remaining amount of Azone in the skin and the amount of 5-FU that permeated across the skin was observed ($r=0.986$). These results suggest that the penetration-enhancing effect of Azone decreases with time and is reversible.

Keywords——penetration enhancer; percutaneous absorption; 5-fluorouracil; Azone; hairless rat; reversible effect

Recently, penetration enhancers have been investigated widely with the aim of developing transdermal therapeutic systems (TTS). Azone (1-dodecylazacycloheptan-2-one, laurocapram) has been reported as a new penetration enhancer for a number of drugs.[2] Its mechanism of action has been examined, and it may be suitable for clinical use.[3] However, the possiblity of cumulative (or repeated) use of Azone is under discussion, especially for drugs which are used for long-term therapy. In the present study, the duration and reversibility of the penetration-enhancing effect of Azone were investigated by measuring remaining Azone in the skin and permeation of 5-fluorouracil (5-FU) across the excised skin in hairless rats.

Experimental

Materials——5-FU and Azone were kindly supplied by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan) and Nelson-Sumisho Co. (Tokyo), respectively. Poly(acrylic acid) (Carbopol 934) (CP) was purchased from B. F. Goodrich Chemical Co. (Cleveland, OH, U.S.A.). Tetracosane was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals and solvents were of reagent grade quality and were used without further purification.

Animals——Male hairless rats (WBN/kob strain) weighing about 200 g, supplied by Saitama Laboratory Animals (Sugito, Saitama, Japan), were used in all animal experiments.

Preparation of Poly(Acrylic Acid) Gels——CP was dispersed in distilled water and an adequate amount of 10% NaOH solution was added to thicken the CP gel and adjust the pH to 7.0. The concentration of CP in the gel was 0.8%. Azone was finely dispersed in the CP gel at a concentration of 3% to form Azone gel for the pretreatment experiments. 5-FU was added to a separate CP gel at a concentration of 1% to form 5-FU gel for the skin permeation experiments.

Procedure for *in Vivo* Penetration Experiments of Azone into Skin——Two grams of 3% Azone gel with a backing of Cateripad (Nichiban Co., Tokyo) was applied to 24 cm^2 (4.0 × 6.0 cm) of the abdominal skin of rats for 24 h

(Azone dose: 2.5 mg/cm^2, 60 mg/rat) The concentration of 3% was chosen for Azone because the same concentration had been used in emulsion formulations in the previous work.[2c,d] To ensure adequate fixation, an elastic bandage (Elastopore No. 50, Nichiban Co.) was wrapped around the body. A Bollman cage (KN-326, Natsume Seisakusho, Tokyo) was also used. Azone gel was removed and wiped off with warm water (nearly 37 °C) at 24 h after the start of the pretreatment experiments. A limited region of the pretreated skin (4 cm^2) (10 mg of Azone was administered on 4 cm^2 of skin) was excised at 0, 1, 3 and 7 d after a 24 h pretreatment and homogenized with 2 ml of physiological saline to determine the concentration of Azone that remained in the skin.

Procedure for *in Vitro* Permeation Experiments of 5-FU across the Skin——The same pretreatment as mentioned above was carried out with Azone gel (without 5-FU) in separate rats. A donor cap from the vertical diffusion cell set (diameter available for drug permeation: 25 mm) was fixed to the skin of a hairless rat with surgical tissue cement (Aron Alpha, Toa Gosei Chemical Co., Ltd., Tokyo) at 0, 1, 3 and 7 d after 24 h pretreatment with Azone gel. The skin was excised with the cell cap from the rat and clamped to a receiver cell. The receiver compartment was filled with 20 ml of physiological saline and the donor cell was filled with 4 g of CP gel containing 40 mg of 5-FU (5-FU dose: 8.15 mg/cm^2). The skin permeation experiments were done at 37 °C in a water bath. At appropriate times, 100 μl of solution was withdrawn from the receiver compartment for analysis. After sampling, 100 μl of saline was added to the receiver to keep the volume constant.

Analysis of Azone——Concentration of Azone in the skin was determined by gas chromatography (GC) with hydrogen flame ionization detecter (FID). Two milliliters of homogenate (4 cm^2 of skin) was mixed with the same volume of *n*-hexane. The mixture was vigorously shaken for 30 min, then centrifuged for 10 min at 3000 rpm. The organic phase (1.5 ml) from the upper layer was evaporated and the residue was dissolved in *n*-amyl alcohol containing tetracosane as an internal standard at a concentration of 0.1%. Then 2 μl of the sample was injected to a GC column (GC-6A, Shimadzu Co., Ltd., Kyoto, Japan). The conditions of GC were as follows: temperatures in the column, and in the detector and injection port were 267 and 290 °C, respectively. Flow rate of carrier gas (N_2) was 37 ml/min. Type of column packing was 5% OV-101 on silicone (1 m length and 3 mm inner diameter). The detection limit of this GC method is 10 ng/ml.

Analysis of 5-FU——Concentration of 5-FU in the receiver compartment was determined by high-performance liquid chromatography (HPLC) using the method reported previously.[4] The detection limit of this HPLC method is 5 ng/ml.

Results and Discussion

Figure 1 shows the time course of the remaining amount of Azone in the skin at 0, 1, 3 and 7 d after a 24 h pretreatment of hairless rat skin with Azone gel. The amount of Azone immediately after the 24 h pretreatment was about 8 μg/cm^2, which was about 0.32% of the dose. As shown in the figure, the amount of Azone in the skin decreased with time, and the amount of Azone at 7 d after the 24 h pretreatment was very low. It is suggested that more than 99% of the Azone disappeared from the skin within 7 d after the 24 h pretreatment.

Figure 2 shows the time course of the cumulative amount of 5-FU that permeated across the excised skin after pretreatment with Azone gel for 24 h. There is a short lag time followed by an almost linear increase in each case, and the amount and rate of skin permeation of 5-FU decreased with time after pretreatment. The permeation rate of 5-FU across the skin at 7 d after pretreatment was almost the same as in the control (without Azone pretreatment).

Figure 3 shows the relationship between the amount of 5-FU that permeated over 6 h across skin which was excised at 0, 1, 3 and 7 d after 24 h pretreatment with Azone gel and the remaining amount of Azone in the skin at the same times after the pretreatment. A good relationship was found between the two ($r=0.986$).

From these results, it was found that the extent of decrease of Azone content in the skin after pretreatment was proportional to the amount of 5-FU that permeated across the skin after pretreatment.

The facts that (i) most Azone disappeared from the skin at 7 d after the 24 h pretreatment (Fig. 1), (ii) the permeability of 5-FU across the skin which was excised at 7 d after 24 h pretreatment with Azone gel was similar to that of the control (without Azone, without Azone pretreatment) (Fig. 2), and (iii) the permeability of 5-FU across the skin when Azone gel was reapplied (2nd pretreatment) to the same site at 7 d after the first 24 h pretreatment (1st pretreatment) with Azone gel was similar to that across the skin which had been pretreated

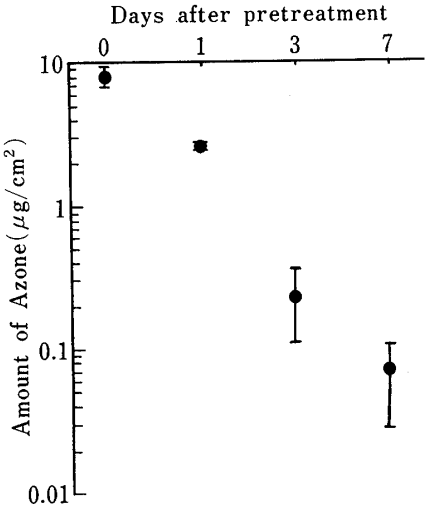

Fig. 1. Amount of Azone in the Skin at 0, 1, 3 and 7 d after a 24 h Pretreatment by Topical Administration of Azone Gel to Rats

Each point represents the mean ± S.E. of 3 animals.

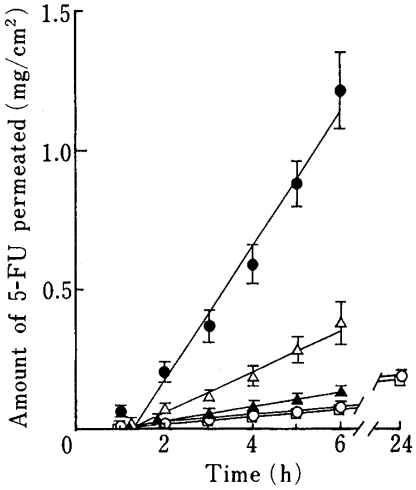

Fig. 2. Effect of Pretreatment with Azone on the Permeation of 5-FU across the Excised Skin

Control (without pretreatment) (○); 0 (●); 1 (△); 3 (▲) and 7 d (□) after 24 h pretreatment with Azone gel. Each point represents the mean ± S.E. of 3 animals.

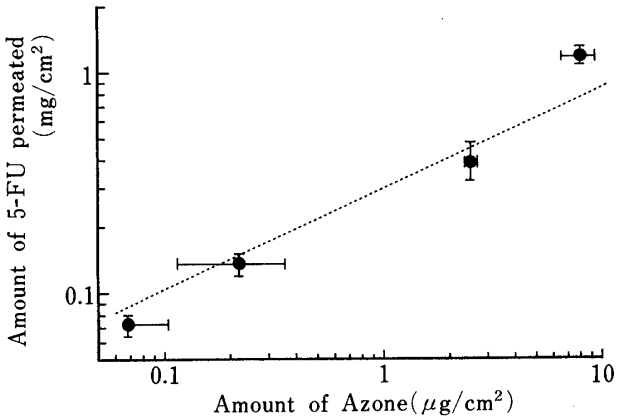

Fig. 3. Relationship between the Cumulative Amount of 5-FU Permeated over 6 h across the Skin and the Remaining Amount of Azone in the Rat Skin

Each point represents the mean ± S.E. Dotted line shows the linear regression curve ($r = 0.986$).

only once (data not shown), suggested that the enhancing effect of Azone was reversible. The reason why the enhancing effect of Azone was reversible would probably be disappearance of Azone itself by exfoliation of the stratum corneum containing Azone and by percutaneous absorption of Azone. Although most Azone may disappear by exfoliation of the stratum corneum, the percutaneous absorption of Azone was confirmed by a tissue distribution experiment using ^{14}C-Azone. A small amount of Azone was distributed mainly to the liver and kidney when administered topically in hairless rats (data not shown). Wiechers *et al.*[5] have studied the percutaneous absorption and elimination of Azone in humans by using a radiolabeled one and reported that Azone was poorly absorbed; trace amounts of radioactivity were found in the urine and feces.

The almost linear relationship of the decreases of Azone concentration in the skin and skin permeation of 5-FU, as shown in Fig. 3, implied that the effect of Azone was dependent on the amount of Azone in the skin. This parameter may be a good index of the enhancing effect of Azone.

In conclusion, development of a TTS containing Azone for cumulative (or repeated) administration may be feasible.

References and Notes

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