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Effect of Azone on the Percutaneous Absorption of 5-Fluorouracil from Gels in Hairless Rats[1)]

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Poly(acrylic acid) gels containing 5-fluorouracil (5-FU) and Azone were prepared and the effects of Azone on 5-FU release from the gels and on 5-FU permeation across the skin were studied by *in vitro* and *in vivo* methods. 5-FU was released rapidly from the gels. The release rate of 5-FU from the gels was higher than that from a commercial ointment. Azone did not affect the *in vitro* drug release from the gels. Experiments on *in vitro* permeation of 5-FU across the hairless rat skin with vertical diffusion cells showed that addition of Azone to the gels markedly enhanced the 5-FU permeability, although a lag time of approximately 6 h was observed. Increasing the Azone concentration in the gels to 15% proportionally increased the permeability of 5-FU. More than 10 h was required to reach a steady-state blood level of 5-FU after administration of 5-FU–Azone gel topically. Pretreatment with Azone, however, shortened the lag time so that a steady-state level was reached sooner. The area under the blood concentration–time curve (*AUC*) after topical administration was comparable to that after oral administration.

These results suggest not only that Azone would be very useful for increasing the skin permeability and blood level of 5-FU, but also that Azone might be useful for developing transdermal therapeutic systems for the delivery of practically unabsorbable drugs.

Keywords——5-fluorouracil; Azone; poly(acrylic acid) gel; drug release; percutaneous absorption; hairless rat

Development of a new drug requires much research, a long development time and also coordinated team efforts of a large group of researchers in various fields. Instead of searching for new drugs using a random, hit-or-miss approach, the development of superior drug delivery systems (DDS), which enhance the therapeutic efficacy of conventional drugs by controlling the release rate and/or targeting to the disease sites, may be an effective approach to improve the efficacy of chemotherapeutic agents.

In the present study, the usefulness of the transdermal route for absorption of antitumor agents was investigated, since the application of transdermal controlled-release medication is one of the most potentially valuable DDS development programs. The effects of vehicles and an absorption enhancer, Azone (laurocapram, 1-dodecylazacycloheptan-2-one, or 1-dodecylhexahydro-2*H*-azepin-2-one), on the permeability of a model antitumor agent, 5-fluorouracil (5-FU), across the hairless rat skin were measured *in vitro* and *in vivo*. Poly(acrylic acid) (Carbopol 934) gels were chosen as a vehicle and compared with a commercial ointment.

Experimental

Materials——Azone was supplied by Nelson Research and Development (Irvine, CA, U.S.A.). 5-FU powder and 5-FU ointment were kindly supplied by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). Poly(acrylic acid) (Carbopol 934) (CP) was purchased from B. F. Goodrich Chemical Co. (Cleveland, OH, U.S.A.). Propylene glycol (PG) and polyethylene glycol (PEG) 400 were purchased from Wako Pure Chemical Industries (Osaka, Japan). All

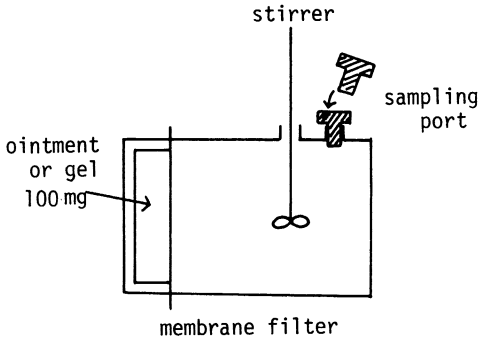

Fig. 1. Apparatus for Drug Release Experiments with a Half Cell

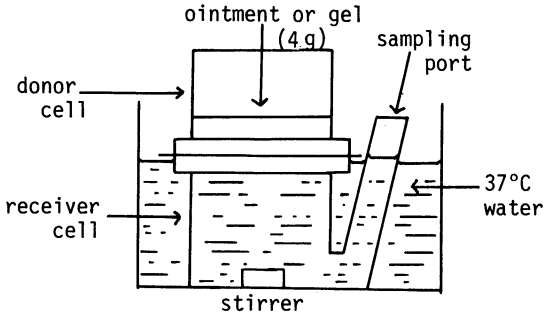

Fig. 2. Apparatus for Drug Permeation Experiments with a Vertical Diffusion Cell

other chemicals were of reagent grade quality and were obtained commercially.

Animals——Male hairless rats (WBN/kob strain) weighing between 150 and 250 g, supplied by Saitama Laboratory Animals (Sugito, Saitama, Japan), were used in all animal experiments.

Preparation of Poly(Acrylic Acid) Gels——CP was dispersed either in water (CP aq gel), PG (CP pg gel), or 50% PEG 400 in water (CP peg gel). In addition, 10% NaOH or diisopropanolamine was added to thicken CP aq gel and CP peg gel or CP pg gel, respectively. The concentration of CP was 0.8% (w/v), and the pH was adjusted to 7.0. 5-FU and Azone were added when required as a solution (or emulsion) in water, PG, or 50% PEG 400 in water. Final concentrations of 5-FU and Azone in gels were 1% and 3—15%, respectively.

Determination of Viscosity of Gels——The viscosities of CP gels and commercial 5-FU ointment were measured with a rotational viscometer (Rotovisko RV 100/M 150, Haake, West Germany) at a shear rate of 0—4650 s^{-1} and 37 °C.

***In Vitro* 5-FU Release from Gels**——The *in vitro* release of 5-FU from CP gels and 5-FU ointment was measured at 37 °C with half of a 2-chamber diffusion cell, as shown in Fig. 1.[3] A membrane filter, (SCWP 02400, pore size 8.0 μm, Millipore Corp., Bedford, MA, U.S.A.) was used to prevent loss of the gel into the sink. The weight of gel was 100 mg and the volume of water in the sink was 2.0 ml. Stirring of the sink solution was carried out with a Teflon propeller driven at 150 rpm with an electric motor. At appropriate intervals, a 100 μl sample was withdrawn from the sink solution for analysis and the same volume of water was added to keep the sink volume constant. Adsorption of 5-FU on the membrane filter was ignored. 5-FU concentration was measured spectrophotometrically at 266 nm.[4]

Procedure for *in Vitro* 5-FU Permeation across Skin——The hair of the abdominal region of hairless rats was carefully shaved with an electric razor, and a donor cap from the vertical diffusion cell set (diameter available for drug permeation: 25 mm) was fixed to the skin with surgical tissue cement (Aron Alpha, Toa Gosei Chemical Co., Ltd., Tokyo). The skin was excised with the cell cap from the rat and clamped to a receiver. The receiver compartment was filled with 20 ml of normal saline and the donor cell was filled with 4 g of ointment. The experiments were done at 37 °C in a water bath. A schematic diagram of the cell set during the experiment is shown in Fig. 2. 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.

Procedure for *in Vivo* 5-FU Percutaneous Absorption——CP aq gel or ointment (2 g) containing 20 mg of 5-FU (1% of total weight) and 60 mg of Azone (3%) with a backing of Cateripad (Nichiban Co., Tokyo) was applied to 22 cm^2 (4.0 × 5.5 cm) of the abdominal skin in rats weighing about 250 g. The concentration of 3% was chosen for Azone in gels because the same concentration had been used in emulsion formulations in the previous work.[5] 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 for the long term experiments. Azone gel (2 g) without 5-FU was applied to the abdominal skin over 24 h in the pretreatment experiments just before the 5-FU–Azone gel was applied. 5-FU–Azone gel was applied to the same site of the abdominal skin after the Azone gel without 5-FU had been wiped off. For comparison purposes, 5-FU was also administered intravenously *via* the tail vein or perorally by gastric intubation in rats. Food was withheld for 24 h before peroral administration. Each dose in the various dosage forms was 80 mg/kg body weight. A 0.5 ml aliquot of blood was withdrawn with a heparinized syringe from the jugular vein at appropriate times for analysis.

Analysis of 5-FU——Concentrations of 5-FU in the receiver compartment and in serum were determined by high performance liquid chromatography (HPLC).

A 100 μl of acetonitrile was added to 100 μl of the receiver solution for deproteinization and mixed. The solution was centrifuged at 16000 rpm at 4 °C for 5 min. Then 25 μl of the resulting supernatant was injected into the HPLC apparatus.

Blood samples were centrifuged at 16000 rpm for 15 min at 4 °C to obtain serum. Then 0.1 ml of 0.5 M NaH_2PO_4, 0.75 ml of water and 4 ml of ethyl acetate were mixed with 0.25 ml of the serum and shaken for 10 min. The same extraction was repeated twice and the supernatant obtained by centrifugation was dried under nitrogen gas. A 100 μl of 1 mM KH_2PO_4 : methanol (9 : 1) was added to the vial and 25 μl of the supernatant obtained by centrifugation was chromatographed through a stainless steel precolumn (30 mm × 4.6 mm, i.d.) and a main column (250 mm × 4.6 mm, i.d.) of microporous silica with octyl and octadecyl chains (RP-8, Braunlee Lab., Inc., Santa Clara, CA, U.S.A. and Nucleosil $10C_{18}$, Nergel, West Germany, respectively). The eluting solvent was 1 mM KH_2PO_4–methanol (9 : 1) and the flow rate was 1.0 ml/min. Detection was done by ultraviolet (UV) absorption measurement at 266 nm. A pump control unit (Type 635-s, Hitachi Co.), a UV detector (Type Ubilog-5III, Oyobunko Kiki Co., Ltd., Tokyo) and an integrator (Type 3390A, Hewlett Packard Co., Avondale, PA, U.S.A) were used.

Results

Effect of Azone on the Rheological Properties of Gels and on *in Vitro* 5-FU Release from Ointment and Gels

The rheological properties of CP gels were found to depend on the kind and amount of neutralizing agents and the concentration of CP. In the present study, we adjusted the concentration of CP and pH to 0.8% and pH 7. Figure 3 shows the viscosity *versus* shear rate

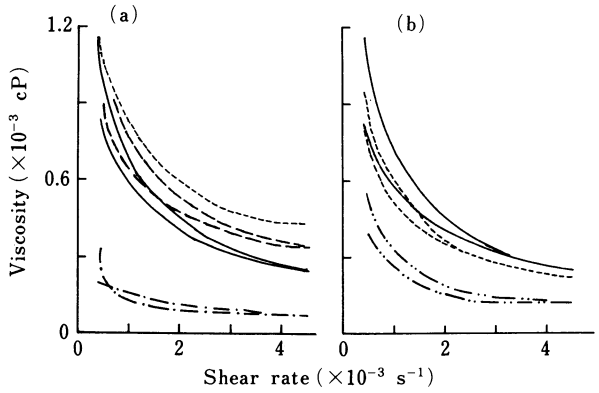

Fig. 3. Rheograms of CP Gels and Ointment

(a) CP aq gel (——), CP pg gel (------), CP peg gel (– – – –) and commercial ointment (— · —).
(b) CP aq gel containing 0% (——), 3% (------) and 10% (– – –) Azone.

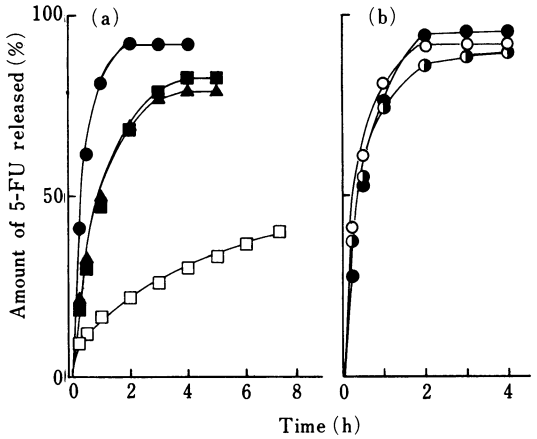

Fig. 4. Effect of Bases on the 5-FU Release from CP Gels and Ointment

(a) CP aq gel (●), CP pg gel (▲), CP peg gel (■) and commercial ointment (□).
(b) CP aq gel containing 0% (○), 3% (◑) and 10% (●) Azone.
Each point represents the mean of 3 experiments.

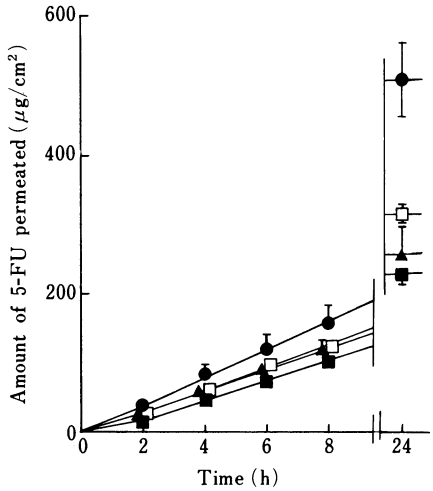

Fig. 5. Effect of Bases on the 5-FU Permeation across Skin

CP aq gel (●), CP pg gel (▲), CP peg gel (■) and commercial ointment (□).
Each point represents the mean ± S.E. of 5 experiments.

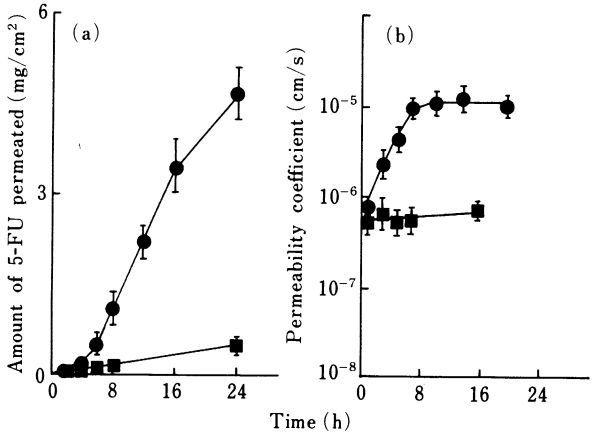

Fig. 6. Effect of Azone on the 5-FU Permeation across Skin

(a) Time course of cumulative amount of drug permeated with (●) and without (■) Azone.
(b) Time course of P; symbols as in (a).
Each point represents the mean ± S.E. of 5 experiments.

curves of a commercial ointment and CP gels. Every gel and ointment showed non-Newtonian flow. Thixotropy was observed, except for CP pg gel. The viscosity of CP pg gel was the highest, whereas that of the ointment was the lowest (Fig. 3a). Addition of Azone to CP aq gel (3% and 10%) decreased the viscosity (Fig. 3b).

In vitro release rates of 5-FU from gels were very fast and most of the drug was released from the gels within 2 h, as shown in Fig. 4. The release rate from the commercial ointment was slower than those from CP gels (Fig. 4a). Addition of Azone to CP gels had no effect on the release properties of 5-FU (Fig. 4b).

5-FU Permeation across Skin from Ointment and Gels

Figure 5 shows the effect of bases on the *in vitro* 5-FU permeation acorss skin. Among 3 kinds of gels, the permeation from CP aq gel was the fastest. The amounts of 5-FU that permeated during the initial 24 h were 504, 258 and 229 $\mu g/cm^2$, and the average permeability coefficients[5] were calculated to be 6.11, 3.09 and 2.72×10^{-7} cm/s for CP aq gel, pg gel and peg gel, respectively. In spite of the higher drug content (5%) in the commercial ointment compared to the gels (1%), the cumulative amount of drug that permeated from the ointment (312 $\mu g/cm^2$) over 24 h was smaller than that from CP aq gel (504 $\mu g/cm^2$). These results suggest the usefulness of CP gels, especially CP aq gel, for 5-FU topical therapy. Only CP aq gel was used for the following experiments.

Effect of Azone on 5-FU Permeation across Skin

Figure 6a shows the time course of 5-FU that permeated across the skin from CP aq gel containing Azone at a concentration of 10%. A marked enhancing effect of Azone on 5-FU permeation was observed. Cumulative amounts of 5-FU that permeated over 24 h were 4.68 mg/cm^2 with Azone and 0.50 mg/cm^2 without Azone. A lag time of approximately 6 h was seen with Azone treatment, which is almost the same as that observed in the case of Azone emulsion system.[5] The permeation rate was decreased after 16 h compared to that during 6—16 h, and this can be explained in terms of the decrease of 5-FU concentration in the gels due to the permeation of more than 50% of the drug into the receiver cell through the skin.

Figure 6b shows the time course of the permeability coefficients.[5] The steady-state permeabilities were 1.3×10^{-5} and 6.1×10^{-7} cm/s with and without Azone, respectively, a difference of about 20 times.

Figure 7 shows the effect of Azone concentration on the *in vitro* permeation of 5-FU across the skin. Increasing Azone concentration in the gels increased 5-FU permeation. After reaching a steady-state level,[5] the permeability *versus* Azone concentration curve was almost

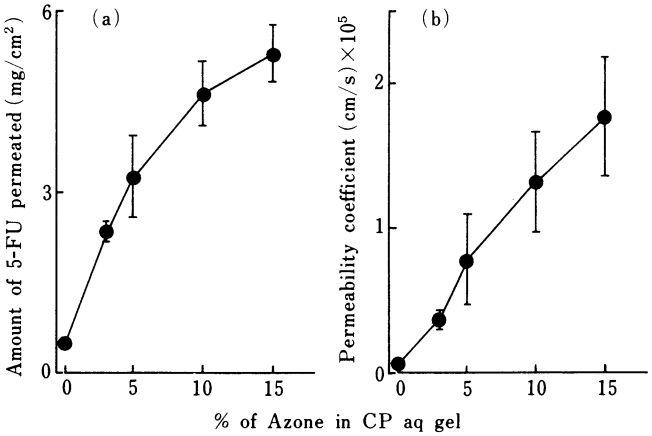

Fig. 7. Effect of Concentration of Azone on the 5-FU Permeation across Skin

(a) Cumulative amount of drug permeated over 24 h; (b) P. Each point represents the mean ± S.E. of 5 experiments.

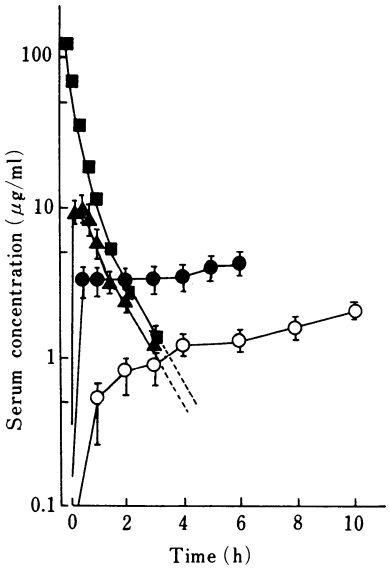

Fig. 8. Time Course of 5-FU Serum Level after Administration of Several Dosage Forms

Dose; 80 mg/kg.
i.v. (■), *p.o.* (▲), topical application with (●) and without (○) pretreatment.
Each point represents the mean ± S.E. of 5 rats.

liner, whereas the curve of the cumulative amount of 5-FU permeated over 24 h against 5-FU concentration in gels was not. The reason is that higher Azone concentrations cause a faster decrease of 5-FU concentration in gels. If a drug-suspending gel was used, straight lines should be obtained for both the cumulative drug transport and permeability data.

Comparison of 5-FU Serum Levels after Topical Administration with Those after i.v. and *p.o.* Administrations

5-FU gel containing Azone at a concentration of 3% was applied to the abdominal skin of rats and the 5-FU serum level after topical application was compared with those after intravenous (i.v.) and *peroral* (*p.o.*) administrations.

After application of 5-FU–Azone gel without Azone gel pretreatment, the serum concentration of 5-FU increased up to 10 h, as shown in Fig. 8. In contrast, a steady-state blood level of about 3—4 μg/ml was found immediately after application of 5-FU–Azone gel onto abdominal skin which had been pretreated with Azone gel without 5-FU for 24 h before

TABLE I. Comparison of AUCs

Route of administration	AUC_{0-6h} ($\mu g\,ml^{-1}\,h$)
i.v. injection	62.41
p.o. administration	13.64
Topical applications	
Without pretreatment	5.22
With pretreatment	20.12

Each value was calculated from the average serum concentrations of 5 rats as shown in Fig. 8.

the drug permeation experiment. 5-FU was not detectable in blood after topical application of CP aq gel without Azone, or commercial ointment. On the other hand, the same dose (80 mg/kg) given i.v. and *p.o.* resulted in a much higher peak concentration, but drug elimination was also much faster than after topical administration of 5-FU–Azone gel.

Table I compares the area under the serum concentration *vs.* time curves (AUCs) for 0—6 h after i.v., *p.o.* and topical administrations. AUC_{0-6h} after topical application without pretreatment was 5.22 $\mu g\,ml^{-1}\,h$. AUC_{0-6h} after topical application with pretreatment was 20.12 $\mu g\,ml^{-1}\,h$. AUC_{0-6h} values after i.v. and *p.o.* treatments were 62.41 and 13.64 $\mu g\,ml^{-1}\,h$, respectively. AUC_{3-6h} values for both treatments were calculated by dividing the serum concentration at 3 h by the terminal elimination constant. AUCs for other periods for which serum concentration data were available were calculated by using the trapezoidal rule.

Addition of Azone to CP gel increased the bioavailability of 5-FU and the AUC after topical administration was comparable to that after oral administration, especially in the case of topical administration after pretreatment with Azone gel.

Discussion

In vitro release of 5-FU from CP aq gel was very fast and no significant effect of the viscosity of gels on the release was found. Since Azone did not promote drug release from the gels, the Azone effect on 5-FU transport across the skin should be caused in the skin. We suggested in the previous papers[5] that Azone affects the skin, especially the stratum corneum. However, the mechanism of the skin penetration enhancing effect of Azone is still unknown.

Stoughton[6a] examined the effect of Azone concentration on the skin permeation of erythromycin and sodium fusidate. The penetration of erythromycin was enhanced by Azone in a monotonic fashion, rising to an apparent plateau at concentrations of Azone above 30—40%. In the case of sodium fusidate, a low concentration of Azone produced a 2- to 3-fold enhancement, but higher concentrations showed less enhancement. Stoughton and McClure[6b] also examined the effect of Azone on 5-FU permeation from water and propylene glycol formulations. A maximum effect was observed at 1.8% Azone; higher concentrations (9.0 and 45.0%) were less effective. From these results and the present data, it is clear that the concentration of Azone which is required to produce optimum enhancement varies from one drug and/or formulation to another. Each drug and formulation should be studied over a range of concentrations of Azone in order to optimize skin permeation.

The blood concentration of 5-FU after administration of 5-FU with Azone gel was very high and comparable to that achieved by systemic therapies (i.v. and *p.o.*) at the same dosage level. In general, the steady-state blood level, C_{ss}, after topical application can be estimated by using the following equation;

$$C_{ss}=J/CL \quad (1)$$

where J is percutaneous absorption flux and CL is total clearance. Flux, J, is expressed as follows;

$$J=P\,Cs\,A \quad (2)$$

where P, Cs and A are the permeability coefficient of the drug across the skin, the solubility of the drug in the formulation and its application area.[7] From both equations, P can be written as follows.

$$P=\frac{C_{ss}CL}{CsA} \quad (3)$$

After topical application of Azone gel with pretreatment, C_{ss} was 3.3 μg/ml, as shown in Fig. 8. Total clearance in hairless rats can be calculated as 0.0890 ml/s from the $AUC_{i.v.}$ (62.41 $\mu g\,ml^{-1}\,h$) as shown in Table I and the dose (20 mg per head) by using the following equation.

$$CL=\frac{\text{dose}}{AUC_{i.v.}} \quad (4)$$

A concentration of 1% (10 mg/ml) was used as the initial concentration of 5-FU in formulations in the present experiments. Since the drug concentration in formulations is almost constant throughout the short-term experiments, this initial concentration can be used instead of Cs. A is 22 cm^2. Substituting these values into Eq. 3 yields 1.34×10^{-6} cm/s as the P value in hairless rat skin.

Phillips *et al.*[8] reported that the CL value of 5-FU in humans was 21.6 ml/s. If we suppose that a saturated aqueous gel is administered (Cs is 17 mg/ml at 37 °C)[9] on 100 cm^2 of human skin and that the permeability across the human skin is the same as that across the hairless rat skin, C_{ss} in humans can be calculated as 105 ng/ml according to Eqs. 1 and 2. This blood level is thought to be a clinically effective concentration.[10] The flux, J, in humans, however, was reported to be smaller than that in rats for many drugs.[11] Thus, J in humans may be estimated as 0.1 to 1 times that in rats. In the present *in vivo* experiments, 3% Azone gel was used. From the *in vitro* permeation experiments (Fig. 7), it is suggested that higher Azone concentrations in the gel may produce higher blood concentrations. As a next step, clinical experiments using dermal formulations containing Azone should be carried out.

In conclusion, Azone appears to be effective for enhancing transdermal transport of hydrophilic compounds such as 5-FU. Transdermal systemic treatment with many drugs may be feasible by using Azone in the near future.

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References and Notes

1) Part of this work was presented at the 14th International Congress of Chemotherapy, June 1985, Kyoto, Japan.
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