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Observations on and pharmacokinetic discussion of percutaneous absorption of mefenamic acid

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Summary

Percutaneous absorption of a non-steroidal anti-inflammatory drug, mefenamic acid, was investigated biopharmaceutically using rabbits. The presence or absence of absorption was examined in simple ointment, macrogols ointment, hydrophilic ointment and absorptive ointment, which are listed in the Japanese Pharmacopeia. Favorable absorption was observed in the case of hydrophilic ointment, and it was also revealed that the volume of the intracutaneous reserve was large.

The effects of the concentration of mefenamic acid, site of application, area of application and of additives in the ointments on acceleration of absorption were studied.

When the concentration of mefenamic acid was more than 4.2%, the absorption was favorable. When the ointment was applied to the thigh, abdomen and back in that order, the amount of absorption increased. Although the amount of absorption increased with an increase in the area of application, no definite relationships could be found between the area of application and the amount of absorption. Concerning the additives, nicotinamide and N-methyl-2-pyrrolidone showed a favorable effect to accelerate absorption.

The fraction of drug absorbed to the total drug (F) after intrarectal administration of hydrophilic ointment and absorptive ointment was compared with those after oral administration and percutaneous absorption.

Introduction

Mefenamic acid is a non-steroidal anti-inflammatory, antipyretic analgesic, which is used for relief of postoperative and traumatic inflammation and swelling, antiphl-

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ogistic analgesic treatment of rheumatoid arthritis, and for antipyresis in acute respiratory tract infection (Winder et al., 1962).

Most of the non-steroidal anti-inflammatory drugs on the market are administered orally, therefore, gastrointestinal disorders become an issue as adverse effects (Tsurumi et al., 1973). Among these drugs, mefenamic acid is widely used as an anti-inflammatory drug with a relatively small number of adverse effects (Tsurumi et al., 1973). In trying to avoid side-effects, administration by injection, percutaneous absorption and rectal absorption have been considered, but there have been no reports on percutaneous absorption of mefenamic acid.

Percutaneous absorption using rabbits as a means of avoiding gastrointestinal disorder was studied pharmacokinetically.

Percutaneous absorption has many external factors that affect the absorption, namely, different types of ointment bases (Naito and Tsai, 1981), the moisture of the skin surface (Naito and Tsai, 1981), site of application (Tsai and Naito, 1982) and area of application (Tsai and Naito, 1982). It is necessary to clarify their relationships in order to obtain favorable absorption.

Kinetic investigations of the effects of the base on percutaneous absorption of mefenamic acid were undertaken using hydrophilic ointment and absorptive ointment, followed by a study of the effects of additives, site of application, area of application and concentration of mefenamic acid. Additionally, intracutaneous retention of mefenamic acid, its decomposition by intracutaneous enzymes and release of mefenamic acid from ointment were studied.

Materials and Methods

Drugs and reagents. Mefenamic acid (JPX), ibuprofen (JPX), acetonitrile, chloroform, KH_2PO_4, H_3PO_4, NaH_2PO_4, Na_2HPO_4, $HClO_4$, NaCl, KCl, NaOH, urea, nicotinamide, N-methyl-2-pyrrolidone, stearyl alcohol, cetyl alcohol, sorbitane sesquioleate, Brij 35, propylene glycol [1] and white petrolatum [2] were used.

Quantitative determination of mefenamic acid.

Total mefenamic acid

Water (1.0 ml) and 10% perchloric acid (1.0 ml) were mixed with a plasma sample (1.0 ml) and incubated for 1 h at 37 ± 2°C. Chloroform (8.0 ml) was added, and the mixture was shaken for 15 min and centrifuged for 10 min at 3000 rpm. An organic layer was filtered through filter paper, and 3.0 ml of the layer were evaporated to dryness. Internal standard solution (100 μl) was added to the residue and dissolved well. Five μl of the solution were injected into a column and determined quantitatively by high-speed-performance liquid chromatography (HPLC).

[1] Nakarai Chemicals, Kyoto, Japan.
[2] Maruishi Pharmaceuticals, Osaka, Japan.

Free mefenamic acid

Water (1.0 ml) and chloroform (8.0 ml) were added to a plasma sample (1.0 ml), and then sodium chloride (1.5 g) was added. After shaking the mixture for 15 min, it was centrifuged for 10 min at 3000 rpm. An organic layer was filtered through filter paper, and 3.0 ml of the organic layer were evaporated to dryness. The residue was examined quantitatively by HPLC.

Cutaneous reserve (mefenamic acid in the skin)

The removed skin, from the horny layer of the epidermis to the dermis, were finely sectioned, water (10 ml) was added, and the mixture was homogenized with a glass homogenizer for 15 min. Total mefenamic acid in 1 ml of the mixture was determined quantitatively and was expressed by the weight per area ($\mu g/cm^2$).

High-speed-performance liquid chromatography

SPD-2A, a UV detector (Shimadzu) and a Shimadzu LC-3A high-speed-performance liquid chromatograph with a Zorbax, ODS column (15 cm × 4.6 mm i.d.) (Shimadzu) were used. In the counter-current distribution method (Dusci and Hackett, 1979), a mixture of acetonitrile and 20 mM phosphate buffer solution (pH = 3.0) (6 : 4 v/v) was used. The column temperature was 30°C, speed was 0.8 ml/min, column pressure was 50 kg/cm^2, wavelength determined was 225 nm, sensitivity was 0.16 aufs and the speed of the chart was 2.5 mm/min.

With regard to the phosphate buffer solution used in the counter-current distribution method, the pH was adjusted to 3.0 by adding phosphoric acid to 20 mM KH_2PO_4. Ibuprofen, whose concentration was adjusted to 100 $\mu g/ml$ by dissolving acetonitrile was used as an internal standard solution.

Mefenamic acid solution for intravenous administration

1 N NaOH solution (0.25 ml) and 1.0 ml of distilled water for injection were added to mefenamic acid (50 mg) and heated at 60°C to be dissolved. The solution was pH 8.0.

Mefenamic acid solution for oral administration

1 N NaOH solution (1.0 ml) and 9.0 ml of distilled water were added to mefenamic acid (200 mg) and heated at 60°C to be dissolved. The solution was pH 8.0.

Preparation of ointment

When hydrophilic ointment is used as the base

With regard to the water phase of the hydrophilic ointment (JPX), 1 N NaOH solution (0.85 ml) and water (2.5 ml) were added to mefenamic acid (200 mg) and the pH was adjusted to 7.42. The additives were dissolved in the water phase.

White petrolatum (25%), stearyl alcohol (22%), propylene glycol (12%) and sodium lauryl sulfate (1.5%) were added to the oil phase.

TABLE 1

PREPARATION OF HYDROPHILIC OINTMENT (JPX)

Serial no.	pH value of aqueous phase	Concentration of MFA [a] (%)	Additives	Applied area (cm^2)	Applied site
B.1	7.42	1.4	None	60	Abdomen
B.2	7.42	2.8	None	60	Abdomen
B.3	7.42	4.2	None	60	Abdomen
B.4	7.42	2.8	None	30	Abdomen
B.5	7.42	2.8	None	90	Abdomen
B.6	7.42	2.8	None	60	Dorsal surface
B.7	7.42	2.8	None	60	Thigh
B.2.a	7.42	2.8	Urea 0.5%	60	Abdomen
B.2.b	7.42	2.8	Urea 2.0%	60	Abdomen
B.2.c	7.42	2.8	NA [b] 0.5%	60	Abdomen
B.2.d	7.42	2.8	NA [b] 2.0%	60	Abdomen
B.2.e	7.42	2.8	NMP [c] 0.5%	60	Abdomen
B.2.f	7.42	2.8	NMP [c] 2.0%	60	Abdomen

[a] Mefenamic acid; [b] nicotinamide; [c] N-methyl-2-pyrrolidone.

The oil and water phases were both heated in a water bath at 75°C, and then the water phase was mixed with the oil phase, stirred at 200–300 rpm with a glass stirring rod and cooled down to room temperature.

In the experiments investigating the relationship between the amounts of absorption and the concentrations of a principal component in the ointment, the pH of the water phase was adjusted to 7.42 by varying the amount of 1 N NaOH solution and the detail conditions were shown in Table 1.

When absorptive ointment is used as the base

With regard to the water phase of the absorptive ointment (JPX), the pH was adjusted to 7.42 by adding 1 N NaOH solution (0.84 ml) and water (2.0 ml) to mefenamic acid (200 mg).

White petrolatum (40%), cetyl alcohol (18%), sorbitane sesquioleate (5%) and Brij 35 (0.5%) were added to the oil phase.

TABLE 2

PREPARATION OF ABSORPTIVE OINTMENT (JPX)

Serial no.	pH value of aqueous phase	Concentration of MFA [a] (%)	Additives	Applied area (cm^2)	Applied site
A.1	7.85	2.8	None	60	Abdomen
A.2	7.42	2.8	None	60	Abdomen

[a] Mefenamic acid.

The oil and water phases were both heated in a water bath at 75°C, and then the water phase was mixed with the oil phase, stirred at 200–300 rpm with a glass stirring rod and cooled to room temperature.

In the experiments investigating the relationship between the pH of the water phase of absorptive ointment and the amount of absorption of a principal component, the pH of the water phase was adjusted by varying the amount of 1 N NaOH solution (Table 2).

Additives

The presence or absence of additives to accelerate absorption of mefenamic acid was determined by adding nicotinamide and N-methyl-2-pyrrolidone to the water phase of the ointment.

Experimental animals. Each group consisted of 5 male rabbits (weighing 1.8–2.2 kg) that were fasted for 24 h before use in the experiment.

Routes of administration

Intravenous administration

Mefenamic acid solution (1.25 ml) (25 mg/kg of body weight) was administered via the auricular vein during a 5-s period.

At 0, 5, 10, 15, 20, 25, 35 and 45 min and at 1, 2, 3, 4 and 5 h after administration, blood samples were collected from the carotid artery. Plasma samples were obtained by centrifugation at 3000 rpm. Catheters, syringes and centrifugation tubes used for the blood sampling were washed with a physiological saline solution containing 10% sodium heparin before use.

Oral administration

Mefenamic acid solution (10 ml) (100 mg/kg of body weight) was injected into the stomach with a No. 9 Nelaton's catheter, and then the inside of the catheter was washed three times with 5 ml of water. Blood samples were collected according to the same method as that used with intravenous administration at 0, 15, 30 and 45 min and at 1, 1.5, 2, 3, 4, 5, 6, 8 and 10 h after oral administration.

Local administration

The hair of the rabbits was shaved with electric clippers taking care not to damage the skin. Twenty-four hours later, the ointment was applied on the abdomen, back or the thigh. The area of application was 5×6, 6×10 or 9×10 cm^2. The ointment (7 g) (100 mg/kg of body weight) was spread on a lint byssus, upon which a thin plastic film was placed, and the area around it was fastened with a tape (occlusive dressing technique, ODT). Blood samples were collected according to the same method as that used with the intravenous injection at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h after administration.

Intrarectal administration

The ointment (3.5 g) (50 mg/kg of body weight) with the same composition as that used with the local administration was inserted into the rabbit rectum with a

syringe, and then the anus was fastened with clips. Blood samples were collected according to the same method as that used with the intravenous injection at 0, 15 and 30 min and at 1, 1.5, 2, 3, 4, 5, 6 and 7 h after administration.

Retention in the skin. Four or 9 h after application of hydrophilic ointment on the rabbit abdomen (ODT), the ointment was cleansed with lintine 200 times, and the dermis of the site (1×2 cm^2) was collected. Three sections were collected from each rabbit.

Decomposition of mefenamic acid with intradermal enzyme

Na_2HPO_4–NaH_2PO_4 buffer solution

0.1 M sodium phosphate dibasic solution was added to 0.1 M disodium phosphate solution and the pH was adjusted to 7.4.

Crude enzyme extract fluid (Misaki et al., 1982)

The hair of the rabbit abdomen was shaved with electric clippers, and an area of about 50 cm^2 was excised. It was frozen at -70°C and slices were prepared quickly. After that, 1.15% (w/v) potassium chloride solution of 18 times the weight of skin was added, and the mixture was homogenized with a glass homogenizer for 15 min in the cold (with ice). Next, it was centrifuged at 0°C at 10,000 rpm for 20 min, and the supernatant fraction was used as crude enzyme extract fluid.

Enzyme reaction

The substrate solution (100 μg/ml) (3.0 ml) in which mefenamic acid was dissolved in 0.1 M Na_2HPO_4-NaH_2PO_4 buffer solution (pH = 7.4) was put in a small stoppered test tube and maintained in a water bath at 37 ± 2°C for 5 min. Crude enzyme extract solution (2.0 ml) was added, and the mixture was shaken immediately for observing the reaction. Twenty-four hours later, 1.0 ml of the reaction solution was taken up and quantitatively determined for total mefenamic acid. A 1.15% (w/v) potassium chloride solution instead of crude enzyme extract fluid was used as the control.

Release of the principal component from ointment (in vitro) (Shiozaki et al., 1982)

Ointments and additives

Hydrophilic ointment (JPX) and absorptive ointment (JPX) were used. Urea, nicotinamide and N-methyl-2-pyrrolidone were used as additives.

Film

Visking seamless cellulose tubing (Visking, Co., site 30/32) was washed with distilled water at 80°C for 2 h before use.

Test solution

0.1 M Na_2PO_4–NaH_2PO_4 buffer solution (pH = 7.4) was used.

Experimental method

One side of a glass cuvette with a capacity of about 15 ml was filled with ointment, and a Visking cellulose tubing (size 30/32) was welded to the leveled surface of the ointment. It was held with the other side of the cuvette and fixed with clamps. A fixed amount of the test solution was put in the cuvette and maintained at $37 \pm 2°C$. Test solution (0.5 ml) was collected every hour for 5 h, and the same amount of test solution was supplemented after the collection. The drug transferred into the test solution was determined by absorption of the ultraviolet part using a Shimadzu electro-spectro-photometer QV-50 at 285 nm.

Results and Discussion

In order to determine the various parameters necessary for discussing percutaneous absorption of mefenamic acid pharmacokinetically, mefenamic acid (25 mg/kg of body weight) was intravenously injected into rabbits. From the curve of concentration in plasma shown in Fig. 1, mefenamic acid was shown to follow (Scheme 1) a two-compartment model. Accordingly, parameters of a pharmacokinetic model of mefenamic acid were determined from the graph (Table 3). Since mefenamic acid showed protein binding in part of the plasma (Ikeda et al., 1979 and Vinnikova et al., 1980), concentrations in plasma of free mefenamic acid were also determined (Fig. 1). As shown in Fig. 1A, the rate of protein binding of mefenamic acid was not so high.

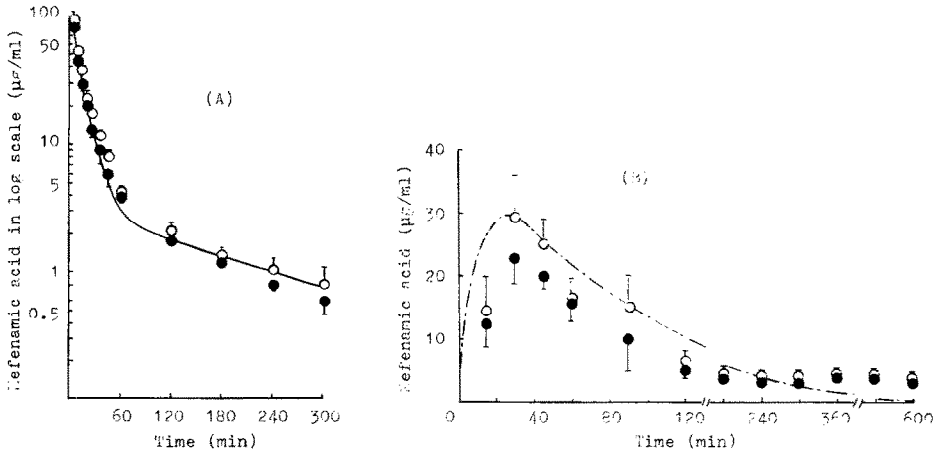
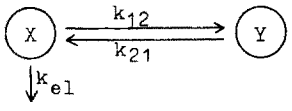


Fig. 1. Mefenamic acid concentration in plasma–time course. A: after intravenous administration of mefenamic acid solution (25 mg/kg). B: after oral administration of mefenamic acid solution (100 mg/kg). Key: O, total mefenamic acid; ●, free mefenamic acid. Solid line shows calculated curve for total mefenamic acid obtained from Eqn. 1 in Scheme 1. Broken line shows calculated curve for total mefenamic acid obtained from Eqn. 1 in Scheme 2. Each point represents the mean value with the standard error.

Scheme 1. Mathematical model for intravenous administration of mefenamic acid

Drug in plasma Drug in tissue



$$X = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$$

$$A = \frac{D\,(k_{21} - \alpha)}{Vc(\beta - \alpha)} \qquad B = \frac{D\,(k_{21} - \beta)}{Vc(\alpha - \beta)}$$

X, drug concentration in plasma
D, dose of intravenous administration
k_{12}, rate constant[1] from central to tissue compartment
k_{21}, rate constant[1] from tissue to central compartment
k_{el}, elimenation rate constant[1]
α, β, pharmacokinetic parameters[1]

[1] See Table 3

The curve of concentrations in plasma of free and total mefenamic acid after oral administration of mefenamic acid (200 mg per animal) is shown in Fig. 1B. As can be seen, the concentration in plasma of mefenamic acid reached a peak 30 min after administration and disappeared completely from the blood 10 h after administra-

TABLE 3

PARAMETERS OF A PHARMACOKINETIC MODEL OF MEFENAMIC ACID IN RABBITS FOLLOWING INTRAVENOUS ADMINISTRATION (25 mg/kg)

Parameter	Value
α	5.59 h^{-1}
β	0.29 h^{-1}
$t_{1/2}$	2.39 h^{-1}
k_{el}	4.03 h^{-1}
k_{12}	1.44 h^{-1}
k_{21}	0.40 h^{-1}
V_c	329 ml
V_t	1184 ml

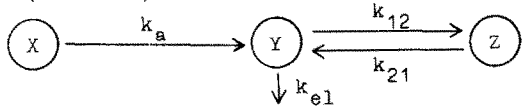
α and β are hybrid first-order rate constants and $t_{1/2}$ is the half-life associated with the terminal exponential process; k_{el} = elimination rate constant from the central compartment; k_{12} = rate constant from the central to tissue compartment; k_{21} = rate constant from the tissue to central compartment; V_c = distribution volume of the central compartment; and V_t = distribution volume of the tissue compartment.

tion. Whether the decline that appeared 60 min after administration was derived from the enterohepatic circulation or from the other factors is under investigation. The rate of protein binding was not so high as with the intravenous administration. Therefore, total mefenamic acid alone was determined in the following experiment, since it is appropriate for considering the rate pharmacokinetically to determine the concentration of total mefenamic acid.

Scheme 2. Mathematical models

(A) Oral or rectal administration of mefenamic acid

Drug in G-I tract (rectum) — Drug in plasma — Drug in tissue



$$Y = P \cdot e^{-\alpha t} + Q \cdot e^{-\beta t} + R \cdot e^{-k_a t}$$

$$P = \frac{k_a FD}{Vc}\left[\frac{(k_{21}-\alpha)}{(k_a-\alpha)(\beta-\alpha)}\right] \quad Q = \frac{k_a FD}{Vc}\left[\frac{(k_{21}-\beta)}{(k_a-\beta)(\alpha-\beta)}\right]$$

$$R = \frac{k_a FD}{Vc}\left[\frac{(k_{21}-k_a)}{(\alpha-k_a)(\beta-k_a)}\right]$$

Y, drug concentration in plasma

D, dose of oral or rectal administration

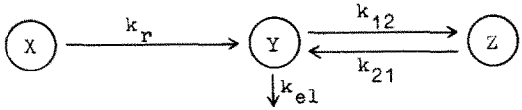
F, fraction of drug absorbed to oral or rectal dose

k_a, absorption rate constant

α, β, k_{12}, k_{21}, k_{el}, Vc, pharmacokinetic parameters[1]

(B) Percutaneous absorption

Drug in ointment — Drug in plasma — Drug in tissue

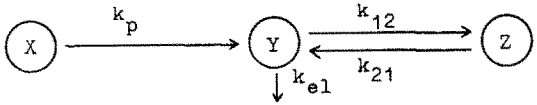


k_r, drug release rate constant

Another symbols are the same as (A)

(C) Percutaneous absorption

Drug in the corium — Drug in plasma — Drug in tissue



k_p, drug release rate constant from corium to plasma

Another symbols are the same as (A)

[1] See Table 3

A theoretical curve in accord with an observed value was calculated by substituting a spontaneous dose and K_a, to the formula (1) of Scheme 2A from the curve of concentration in plasma after oral administration. When the dose was 85.5 mg and K_a was 0.85 h^{-1}, the theoretical curve was almost the actual value, but slight slippage was observed.

The F value was calculated as follows:

$$F = \frac{\text{the amount actually absorbed}}{\text{dose}}$$

F after oral administration was 42.8%.

Among the ointment bases in the Japanese Pharmacopeia (JPX), mefenamic acid was not absorbed into the blood in the case of macrogols or simple ointment.

Absorptive ointment was slightly absorbed and hydrophilic ointment was favora-

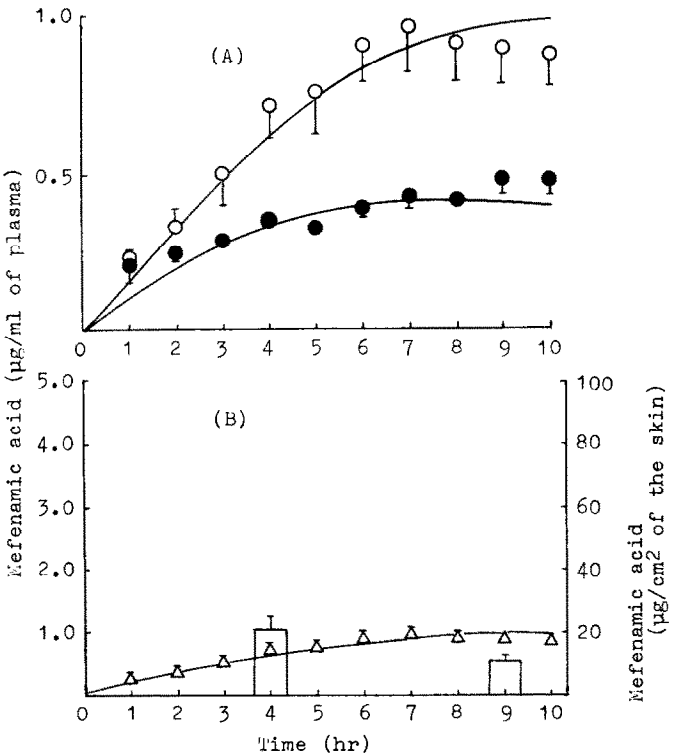


Fig. 2. Mefenamic acid concentration. A: effect of pH on the percutaneous absorption of mefenamic acid from absorptive ointment (ODT). Key: ●, aqueous phase, pH 7.85 (No. A.1 in Table 2); ○, aqueous phase, pH 7.42 (No. A.2 in Table 2). B: cutaneous reserve and plasma concentration with percutaneous absorption of mefenamic acid from absorptive ointment. Key: △, plasma concentration; column, cutaneous reserve. All curves for mefenamic acid were calculated from Eqn. 1 in Scheme 3. Each point and column represent the mean value with the standard error.

TABLE 4

AMOUNTS OF SODIUM HYDROXIDE AND CORRESPONDING pH VALUES FOR THE AQUEOUS PHASE, AND PERCENTAGES OF UNDISSOCIATED MEFENAMIC ACID [a] IN THE AQUEOUS PHASE AT EACH pH

Sodium hydroxide (mg/7 g of ointment)	Aqueous phase pH	Percent of undissociated mefenamic acid in the aqueous phase at 25°C [b]
33.6	7.42	0.06
34.8	7.85	0.02

[a] The pK_a value of mefenamic acid (4.20) was obtained by Winder et al. (1962).
[b] The value was determined from the Henderson-Hasselbach equation.

TABLE 5

RATIO OF THE AREA UNDER THE PLASMA CONCENTRATION VS TIME CURVE (AUC) AFTER TOPICAL AND RECTAL ADMINISTRATION OF MEFENAMIC ACID TO THE ORAL ADMINISTRATION

Serial no. [a]		Mean t_{max} (h)	Ratio of $AUC_{0\text{-}10}$
200 mg, oral		0.50	1.00 (67.31 ± 6.88 $\mu g \cdot h \cdot ml^{-1}$) [b]
200 mg, topical	(A.2)	7	0.10
	(B.2)	8	0.24
100 mg, rectal	(A.2)	0.33	0.74
	(B.2)	0.33	1.04

[a] See Table 2 and Table 1.
[b] AUC in the case of oral administration.

TABLE 6

RATIO OF THE AREA UNDER THE PLASMA CONCENTRATION VS TIME CURVE (AUC) AFTER TOPICAL ADMINISTRATION OF MEFENAMIC ACID TO THE ORAL ADMINISTRATION [a]

Serial no. [b]	Mean t_{max} (h)	Ratio of $AUC_{0\text{-}10}$
A.1	9	0.05
B.1	7	0.20
B.3	5	0.42
B.4	6	0.27
B.5	8	0.32
B.6	8	0.26
B.7	10	0.10
B.2.a	6	0.23
B.2.b	6	0.30
B.2.c	7	0.26
B.2.d	7	0.34
B.2.e	7	0.36
B.2.f	10	0.34
B.2 [c]	7	0.09

[a] AUC after the oral administration was 67.31 ± 6.88 $\mu g \cdot h \cdot ml^{-1}$.
[b] See Table 1 and Table 2
[c] The ointment was applied without ODT.

bly absorbed. Therefore, curves of concentrations in plasma after percutaneous absorption under various conditions were compared.

Fig. 2A shows the curve of concentrations in plasma when the pH of the water phase of absorptive ointment was adjusted to 7.42 and 7.85, and Table 4 shows the rate of non-dissociable mefenamic acid in the water phase. When comparing areas under the concentrations in plasma (AUC) (Tables 5 and 6), the rate of non-dissociable mefenamic acid with pH 7.42 was twice as high as that with pH 7.85, supporting the report (Tsai and Naito, 1982) that the percutaneous absorption is favorable when the pH of the water phase is low. Therefore, the pH of the water phase of the absorptive and hydrophilic ointments used in the following experiments was always 7.42.

Fig. 2B shows the curve of concentrations in plasma after application of absorptive ointment (by the ODT) and the relationship between concentration in plasma and cutaneous retention with percutaneous absorption of mefenamic acid from

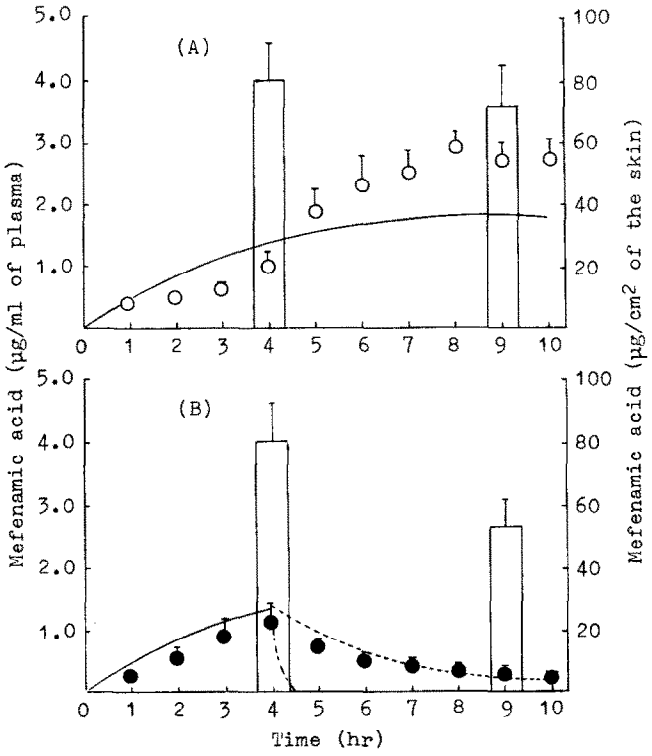


Fig. 3. The relationship between concentration in plasma and cutaneous reserve with percutaneous absorption of mefenamic acid from hydrophilic ointment (ODT). A: the ointment was removed at 10 h after the application. Key: ———, calculated from Eqn. 1 in Scheme 3; ○, plasma level; column, cutaneous reserve. B: the ointment was removed at 4 h after the application. key: ———, calculated from Eqn. 1 in Scheme 3; – · –, calculated from Eqn. 1 in Scheme 1; - - - - - -, calculated from Eqn. 1 in Scheme 2 (model C); column, cutaneous reserve. Each point and column represent the mean value with the standard error.

absorptive ointment at 4 and 9 h after application. It was revealed that percutaneous absorption of mefenamic acid from absorptive ointment was poor. As a result of examination of the presence or absence of additives to accelerate absorption, neither urea, nicotinamide or N-methyl-2-pyrrolidone showed any such effect.

Fig. 3A shows the relationship between concentration in plasma after application of hydrophilic ointment and cutaneous retention with percutaneous absorption of mefenamic acid at 4 and 9 h after application. The degree of increase in the concentration in plasma increased at 4 h after application, and the intracutaneous retention was great 4 and 9 h after application.

Fig. 3B shows the relationship between concentration in plasma of the hydrophilic ointment cleansed away 4 h after application (by the ODT) and intracutaneous reserve 4 and 9 h after application. The concentration in plasma gradually decreased at 4 h after application, and the reserved amount did not decrease so significantly at 9 h after application as compared with that at 4 h.

In Figs. 2B and 3A, it can be seen that intracutaneous reserve was greatly related to percutaneous absorption of mefenamic acid from absorptive and hydrophilic ointment. According to Fig. 2B, it is considered that intracutaneous reserve was primarily observed up to 4 h after application and mefenamic acid released from the cutaneous reservoir is transferred into the blood, and the concentration in plasma increases. Additionally, in Fig. 3A, it can be noted that the mefenamic acid reserved intracutaneously was released poorly into plasma and was reserved in the skin for a long time.

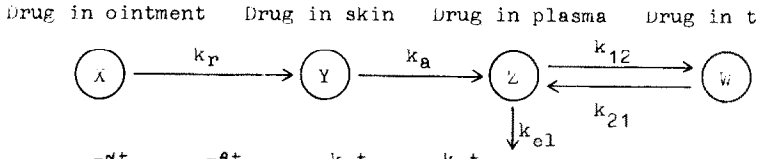
A kinetic model representing percutaneous absorption is generally shown in Scheme 2B, but it is appropriate to consider skin as one fraction because the intracutaneous retention occupies an important position in percutaneous absorption of mefenamic acid. Therefore, the authors adopted the model shown in Scheme 3 to percutaneous absorption of mefenamic acid.

A theoretical curve, which is nearly the actual value of all the ointments tested, was calculated by substituting spontaneous doses, k_r and k_a, to the Eqn. 1 in Scheme 3. Many combinations of k_r and k_a were considered, but k_a was 0.07 h^{-1} in all combinations. From this fact, it was presumed that the constant of absorption rate of mefenamic acid in the skin into the blood is definite in percutaneous absorption, regardless of the kind of ointment base used and the presence or absence of additives in the ointment.

In order to study the effects of the concentration of mefenamic acid in the ointment on percutaneous absorption, ointments containing 1.4% and 4.2% of mefenamic acid were applied to the rabbit abdomen (by the ODT), and the curve of concentration in plasma is shown in Fig. 4A. When comparing AUC according to Tables 5 and 6, there was no difference of AUC between ointment containing 1.4% and that containing 2.8% of mefenamic acid. However, AUC of the ointment containing 4.2% of mefenamic acid was about 1.75 times as high as those of the other two ointments. As can be seen in Table 7, the ointments were compared with regard to release velocity constant (k_r) and F.

The ointment containing 1.4% of mefenamic acid was approximately the same as that containing 2.8% as far as k_r and F were concerned, but k_r and F of the

Scheme 3. Pharmacokinetic model for percutaneous absorption



$$Z = P\,e^{-\alpha t} + Q\,e^{-\beta t} + R\,e^{-k_r t} + S\,e^{-k_a t} \qquad (1)$$

$$P = \frac{k_r k_a F D}{Vc}\left[\frac{(k_{21}-\alpha)}{(\beta-\alpha)(k_r-\alpha)(k_a-\alpha)}\right] \qquad Q = \frac{k_r k_a F D}{Vc}\left[\frac{(k_{21}-\beta)}{(\alpha-\beta)(k_r-\beta)(k_a-\beta)}\right]$$

$$R = \frac{k_r k_a F D}{Vc}\left[\frac{(k_{21}-k_r)}{(\alpha-k_r)(\beta-k_r)(k_a-k_r)}\right] \qquad S = \frac{k_r k_a F D}{Vc}\left[\frac{(k_{21}-k_a)}{(\alpha-k_a)(\beta-k_a)(k_r-k_a)}\right]$$

Z, drug concentration in plasma; D, topical dose; F^a, fraction of drug absorbed to topical dose; k_a^a, absorption rate constant; k_r^a drug release rate constant; α, β, k_{12}, k_{21}, k_{el}, Vc, pharmacokinetic parameters[b]

[a] See Table 7

[b] See Table 3

ointment containing 4.2% were about 1.5 and 1.7 times, respectively, as high as those of the other two ointments. However, the concentration of the principal component was set at 2.8% in the following experiment for the purpose of comparison with the same dose as the oral administration.

In order to study the effects of the area of application on percutaneous absorption, ointment was applied to the areas 5×6 cm^2 and 9×10 cm^2 in size (by the ODT), and a curve of concentrations in plasma was determined (Fig. 4B). As a result of comparison of AUC, as shown in Tables 5 and 6, there was no significant difference among the areas 5×6 cm^2, 6×10 cm^2 and 9×10 cm^2 in size.

In order to study the effects of the site of application on percutaneous absorption, the ointment was applied to the back and the thigh of rabbits (by the ODT), and a curve of concentration in plasma was obtained (Fig. 5A). As a result of comparison of AUC, as can be seen in Tables 5 and 6, there was almost no difference in AUC between the back and the abdomen, while AUC was less than half in the case of application to the thigh. With regard to the relationship between AUC and the site of application, it is reported that AUC increases in the order of the thigh, abdomen and back (Tsai and Naito, 1982). In the present experiment as well, a similar tendency was observed, but there was no significant difference in AUC between the abdomen and the back.

The ODT is a method for obtaining favorable absorption by enhancing the moisture of the skin surface. In order to study the usefulness of this method,

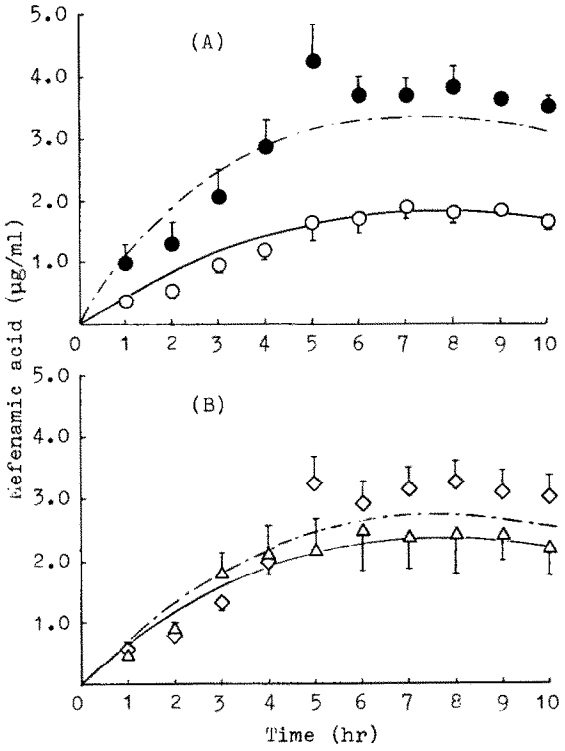


Fig. 4. Mefenamic acid concentration. A: effect of concentration of mefenamic acid in the ointment base on percutaneous absorption from hydrophilic ointment (ODT). B: effect of the size of the applied area in the abdomen on the percutaneous absorption of mefenamic acid from hydrophilic ointment (ODT). Key: ○–○, 1.4% (No. B.1 in Table 1); ●–·–●, 4.2% (No. 5 in Table 1); △–△, 30 cm^2 (No. B.4 in Table 1); ◇–◇, 90 cm^2 (No. B.5 in Table 1). All curves (solid and broken lines) for mefenamic acid were calculated from Eqn. 1 in Scheme 3. Each point represents the mean value with the standard error.

ointment was applied to the rabbit abdomen without ODT, and a curve of concentration in plasma was obtained (Fig. 5B). AUC by the ODT was about 2.5 times as high as that without ODT.

The presence or absence of effects of various additives to accelerate absorption of mefenamic acid from hydrophilic ointment was investigated. Urea, nicotinamide and N-methyl-2-pyrrolidone were used as additives. The amounts of 0.5% and 2.0% of the total amount of ointment were added.

The effects of urea to accelerate percutaneous absorption have already been reported (Naito and Tsai, 1981). It has also been reported that N-methyl-2-pyrrolidone has an effect to enhance the release of a drug from ointment in vitro (Shiozaki et al., 1982).

Curves of concentrations in plasma when urea, nicotinamide and N-methyl-2-pyrrolidone were added to hydrophilic ointment are shown in Figs. 6A, 6B and 7A, respectively. The absorbefacient effects of additives were compared between the ointment with an additive of 2.0% and that with an additive of 1.5% from the AUC

shown in Table 6. No absorbefacient effect was observed in the ointment with urea of 2.0% or 1.5%. When 0.5% of nicotinamide was added to an ointment, no absorbefacient effect was observed, while absorbefacient effects were observed when 2.0% of nicotinamide was added to the ointment; that is, AUC of the ointment with 2.0% of nicotinamide was about 1.5 times as high as that of the ointment without additives.

When N-methyl-2-pyrrolidone of 0.5% and 2.0% was added, absorbefacient effects of about 1.5 times were observed according to the rates of AUC.

F became about 1.5 times as high when absorbefacient effects were observed when nicotinamide of 0.5% and N-methyl-2-pyrrolidone of 0.5% and 2.0% were added. k_r also became about 1.5 times as high as much as that after application of the ointment without additives. From the above results, it was clarified that the absorbefacient effects of these additives were due to the increases in both F and k_r.

Table 8 shows an apparent constant of release velocity (k) of mefenamic acid from hydrophilic and absorptive ointments in vitro (Shiozaki et al., 1982). As shown in Fig. 7B, the apparent constant of release velocity is expressed as the gradient of a straight line when the amount released per unit area at each time was determined by

TABLE 7

PHARMACOKINETIC PARAMETERS ON PERCUTANEOUS ABSORPTION FROM OINTMENTS

Serial no. [a]	K_r [b] (h^{-1})	k_a [c] (h^{-1})	F [d]
A.1	0.30	0.07	0.07
A.2	0.10	0.07	0.25
B.1	0.28	0.07	0.29
B.2	0.24	0.07	0.30
B.3	0.36	0.07	0.51
B.4	0.30	0.07	0.37
B.5	0.30	0.07	0.43
B.6	0.24	0.07	0.30
B.7	0.10	0.07	0.25
B.2.a	0.40	0.07	0.31
B.2.b	0.40	0.07	0.33
B.2.c	0.30	0.07	0.33
B.2.d	0.30	0.07	0.44
B.2.e	0.36	0.07	0.40
B.2.f	0.36	0.07	0.40
B.2 [e]	0.50	0.07	0.11
A.2 [f]	–	1.00	0.66
B.2 [f]	–	1.20	0.99

[a] See Table 1 and Table 2.
[b] Drug release rate constant.
[c] Drug absorption rate constant.
[d] Fraction of drug absorbed to the total drug in ointment bases.
[e] The ointment was applied without ODT.
[f] Rectal administration.

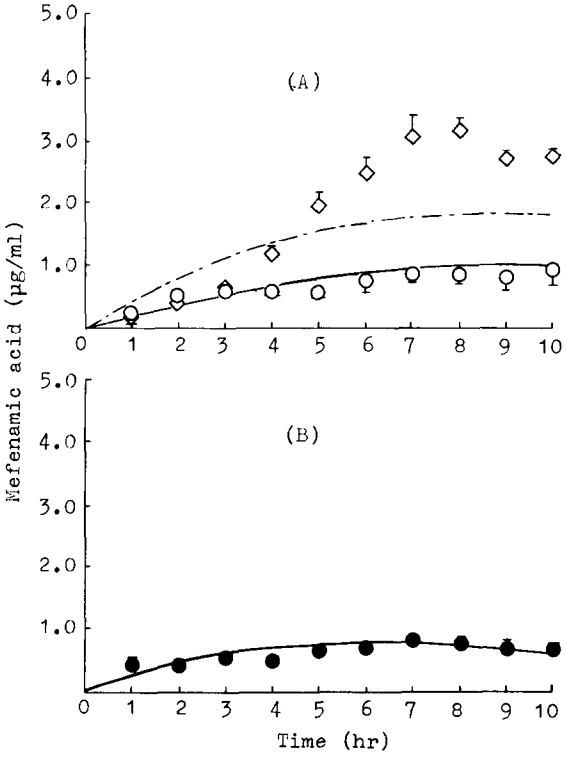


Fig. 5. Mefenamic acid concentration. A: effect of applied skin site on the percutaneous absorption of mefenamic acid from hydrophilic ointment (ODT). B: percutaneous absorption of mefenamic acid in hydrophilic ointment without ODT. Key: ◇·–·◇, dorsal surface (No. B.6 in Table 1); O–O, thigh (No. B.7 in Table 1). All curves (solid and broken lines) for mefenamic acid were calculated from Eqn. 1 in Scheme 3. Each point represents the mean value with the standard error.

TABLE 8

APPARENT RELEASE RATE CONSTANT OF MEFENAMIC ACID FROM HYDROPHILIC OINTMENT (JPX) AND ABSORPTIVE OINTMENT (JPX) CONTAINING VARIOUS ADDITIVES (IN VITRO) [a]

Additives	Hydrophilic ointment		Absorptive ointment	
	$k(\times 10^{-6})$ [b]	ratio [c]	$k(\times 10^{-6})$ [b]	ratio [d]
None	0.395	1.00	0.242	1.00
Urea 2.0%	0.377	0.85	0.249	1.03
Urea 0.5%	0.231	0.58	0.249	1.03
NA [e] 2.0%	0.565	1.43	0.245	1.01
NA [e] 0.5%	0.420	1.06	0.196	0.81
NMP [e] 2.0%	0.510	1.29	0.339	1.40
NMP [e] 0.5%	0.591	1.50	0.351	1.45

[a] Measured by dialysis (Shiozaki et al., 1982).

[b] Apparent release rate constant ($mol/cm^2/h^{1/2}$).

[c] Ratio when the apparent release rate constant of mefenamic acid from hydrophilic ointment without additives was 1.00.

[d] Ratio when the apparent release rate constant of mefenamic acid from absorptive ointment without additives was 1.00.

[e] NA, nicotinamide; NMP, N-methyl-2-pyrrolidone.

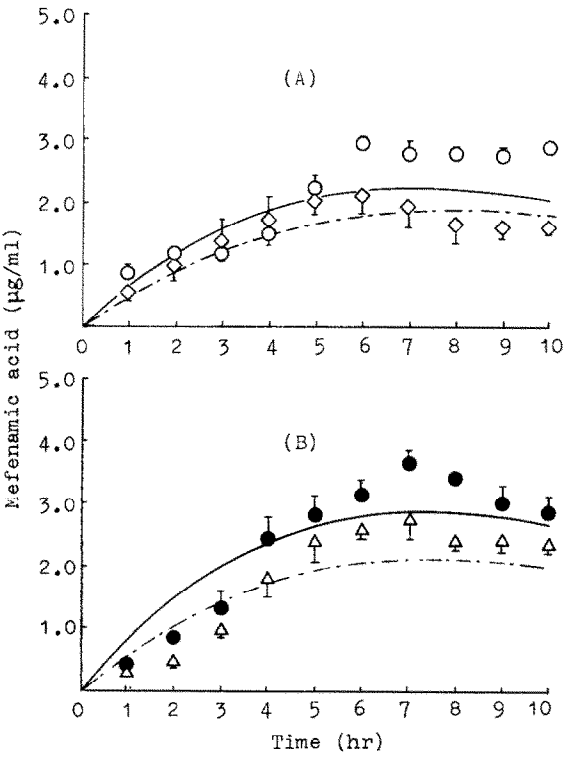


Fig. 6. Mefenamic acid concentration. A: effect of various urea concentrations on the percutaneous absorption of mefenamic acid from hydrophilic ointment (ODT). B: effect of various nicotinamide concentrations on the percutaneous absorption of mefenamic acid from hydrophilic ointment (ODT). Key: ◇·–·◇, 0.5% urea (No. B.2.a in Table 1); ○–○, 2.0% urea (No. B.2.b. in Table 1); △·–·△, 0.5% nicotinamide (No. B.2.c in Table 1); ●–●, 2.0% nicotinamide (No. B.2.d in Table 1). All curves (solid and broken lines) for mefenamic acid were calculated from Eqn. 1 in Scheme 3. Each point represents the mean value with the standard error.

plotting the amount released along the ordinate and plotting the square-root of time along the abscissa. It was revealed that k was in good accordance with AUC in hydrophilic ointment when k of the ointment without additives was 1.00.

Among the absorptive ointments, N-methyl-2-pyrrolidone alone enhanced the k value. On the other hand, the k of a hydrophilic ointment was about 1.6 times as high as that of an absorptive ointment.

Although an in vitro experiment was performed to study breakdown of mefenamic acid by intradermal enzymes under the condition of retention (Misaki et al., 1982), no breakdown was observed. This seems to be due to the structural stability of mefenamic acid as well as to the fact that the principal intradermal enzyme is a hydrolase.

The velocity of transfer of the cutaneously reserved drug to the plasma was determined after application of an absorptive ointment (without additives) for 4 h. With regard to the plasma concentration after the ointment was removed, it

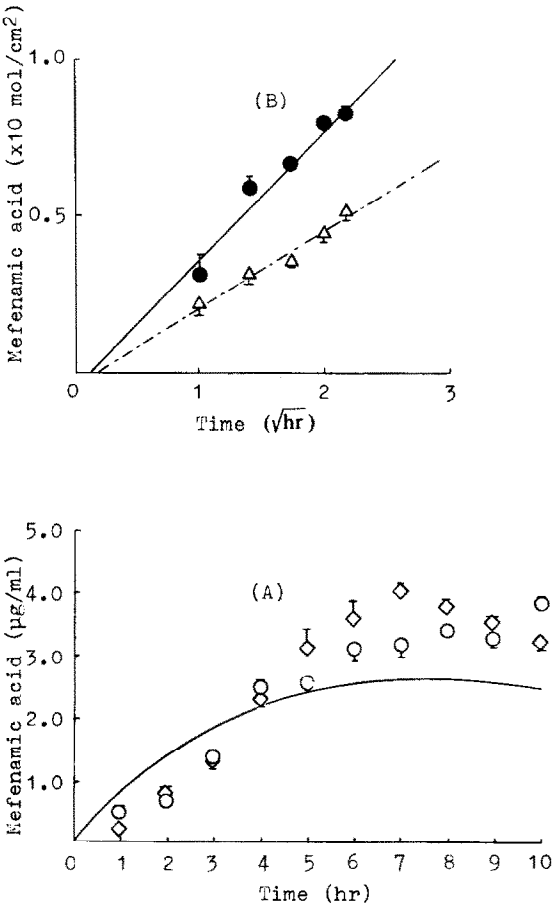


Fig. 7. Mefenamic acid concentration. A: effect of various N-methyl-2-pyrrolidone concentrations on the percutaneous absorption of mefenamic acid from hydrophilic ointment (ODT). Key: ◇–·–◇, 0.5% N-methyl-2-pyrrolidone (No. B.2.e in Table 1); ○–○, 2.0% N-methyl-2-pyrrolidone (No. B.2.f in Table 1); the curve for mefenamic acid was calculated from Eqn. 1 in Scheme 3 and the curve of addition of 0.5% N-methyl-2-pyrrolidone close to observed points was the same as in the case of addition of 2.0% N-methyl-2-pyrrolidone, each point represents the mean value with the standard error. B: release of mefenamic acid from hydrophilic ointment and absorptive ointment. Key: △–·–△, absorptive ointment (No. A.2 in Table 2); ●–●, hydrophilic ointment (No. B.2 in Table 1), each point was plotted according to Higuchi's equation (Shiozaki et al., 1982) and represents the mean value with the standard deviation.

decreased according to the model shown in Scheme 1 if the cutaneously reserved drug was not released (dash–dot line in Fig. 3B). However, the actual values did not agree with the dash-dot line, and mefenamic acid was thought to be released from the skin. When the plasma concentrations were calculated according to the model in Scheme 2C under the condition that the constant of velocity of release from the skin to the plasma (k_p) was set as 0.7 h^{-1}, they agreed with the actual values (broken line

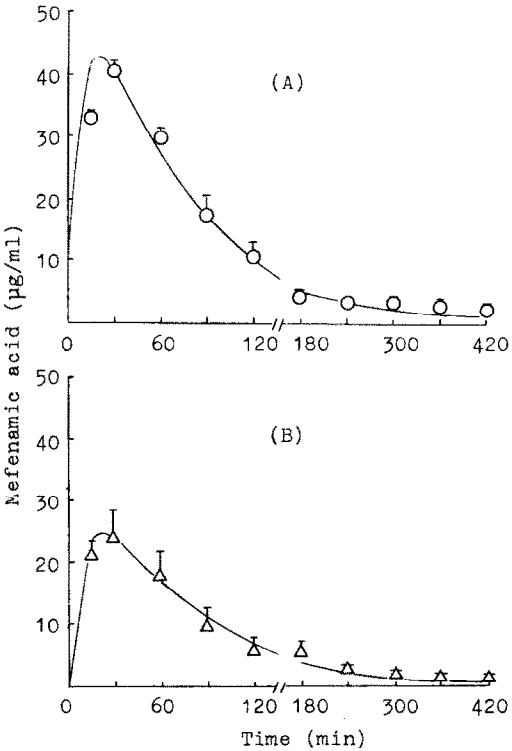


Fig. 8. Mefenamic acid concentration in plasma–time course. A: after rectal administration of hydrophilic ointment (50 mg/kg, No. B.2 in Table 1). B: after rectal administration of absorptive ointment (50 mg/kg, No. A.2 in Table 2). Solid line shows calculated curve obtained from Eqn. 1 in Scheme 2. Each point represents the mean value with the standard error.

in Fig. 3B). These results revealed that mefenamic acid in the skin is transferred to plasma with a rate constant different from k_a and k_r.

In order to compare percutaneous absorption, oral administration and rectal administration, hydrophilic and absorptive ointments were administered intrarectally, and the plasma concentrations of mefenamic acid were determined (Fig. 8A and B). Table 7 shows the k_a and F values.

In this experiment, absorption of an absorptive ointment was favorable although the dose was half of that given by oral administration and percutaneous absorption. The plasma concentration reached a peak about 20 min after administration, about 1.5 times as high as that with oral administration. The F was 99% (Table 7), revealing that the fraction of the hydrophilic ointment absorbed in the total drug is favorable in rectal absorption of mefenamic acid. With regard to the absorptive ointment as well, the fraction was more favorable than that with oral administration although it was lower than that in the absorptive ointment (Table 7).

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