

Pharmacokinetics of Ketotifen Fumarate after Intravenous, Intranasal, Oral and Rectal Administration in Rabbits¹⁾

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The pharmacokinetics of ketotifen fumarate (KF) was examined after administration in rabbits through four different routes (intravenous, intranasal, oral and rectal). The time-course of the plasma concentration of KF after intravenous administration (1 mg/kg dose) fitted a two-compartment open model. KF was rapidly absorbed and showed a high plasma concentration within 0.33 h after intranasal administration. The absolute bioavailability of KF after intranasal administration was 66%. After oral administration at a dose of 1 mg/kg, the plasma concentration of KF was below the detection limit of HPLC analysis. Even at 5 mg/kg, the value of the area under the plasma concentration–time curve (*AUC*) after oral administration of KF was significantly lower than that after intranasal administration of 1 mg/kg. Oral bioavailability was only 8%. The very low bioavailability of KF after oral administration might be due to the first-pass effect in the liver. We also prepared suppositories containing KF (1 mg/kg) for rectal administration in rabbits. After rectal administration, KF was rapidly absorbed and its bioavailability was 34%. These results indicated that the intranasal route appears the most effective for administering KF, and that rectal administration may be superior to oral administration in terms of bioavailability.

Key words ketotifen fumarate; pharmacokinetic; intranasal administration; rectal administration; oral administration; HPLC analysis

Ketotifen fumarate (KF), 4-(methyl-4-piperidylidene)-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-10(9*H*)-one hydrogen fumarate, has been widely used as an anti-allergic and anti-anaphylactic agent in adults and children.^{4–9)} KF has been used orally in the main to prevent allergic asthma by directly blocking the release of the allergic mediator from mast cells. KF is marketed as capsules, dry syrup, syrup, eye drops and nasal solution in Japan. However, there is little published pharmacokinetic data on this agent.^{4,10–12)} In particular, there is little available data on its pharmacokinetic properties after intranasal administration. Most analysis of KF in plasma has been performed by modified GC-MS or radioimmunoassay (RIA).^{10–13)} However, these methods are cumbersome and require expensive specialized equipment. The analysis of KF by an ion-pair HPLC method has been reported.^{14,15)} However, the pretreatment of samples is complicated and quantitative errors are produced occasionally when this method is used.

One of the purposes of this study was to establish a method of HPLC analysis with sufficient sensitivity and simple operation to determine the plasma concentrations of KF. Also, we studied the pharmacokinetics after intravenous, intranasal and oral administrations of KF in rabbits by the HPLC method. We prepared suppositories containing KF for comparison of pharmacokinetic parameters after its oral administration.

MATERIALS AND METHODS

Materials KF powder (lot No. 331000) and KF nasal solution (Zaditen[®] nasal solution, lot No. 104, 6.048 mg/8 ml as KF) were obtained from Novartis Pharma K.K., Tokyo, Japan. Witepsol (Vosco[®] H-15, lot No. 8417) was purchased from Maruishi Pharm. Co., Osaka, Japan. Ethyl *p*-aminobenzoate (lot No. 102D2101), an internal standard (i.s.), was purchased from Kanpan Techno Co., Inc., Tokyo. HPLC-

grade acetonitrile was purchased from Kanpan Techno Co., Inc. All other chemicals were of reagent grade.

Animals Male Japanese albino rabbits (2.7–3.3 kg) from Ichikawa Laboratory (Tokyo) were used. The animals were housed individually in cages under environmentally controlled conditions of illumination (12 h light/dark cycle), humidity (55±5%) and temperature (23±1 °C). They were fasted for 24 h prior to intravenous, nasal and rectal administration of KF. For oral administration, gastric-emptying time controlled rabbits¹⁶⁾ were employed. Namely, the gastric contents of the animals were washed out with saline warmed at 37 °C and the rabbits were muzzled to prevent coprophagy during the night.

Principles in good laboratory animal care were followed and animal experimentation was in compliance with the Guidelines for the Care and Use of Laboratory Animals in Health Sciences University of Hokkaido.

HPLC Conditions for KF The chromatographic system consisted of an LC-10AD pump, a CTO-10A column oven, an SCL-10A system controller, an SIL-10A autoinjector, a C-R6A computing integrator and an SPD-10A UV detector (Shimadzu Co., Ltd., Kyoto, Japan). Separation was performed on a LiChrosorb RP-8 column (5 µm particle size, 250×4.6 mm, i.d.) from Merck (Darmstadt, Germany) and a Mightysil RP-18 GP column (5 µm particle size, 250×4.6 mm, i.d.) from Kanpan Techno. The mobile phase was 50 mM potassium bi-phosphate containing 30% (v/v) acetonitrile. The pH of the mobile phase was adjusted to 4.0 with phosphoric acid. The column temperature was maintained at 40±0.1 °C. The UV detector was set at 295 nm. The concentration of KF was calculated from peak height to internal standard ratio. For *in vivo* and *in vitro* experiments, the flow rate was 0.65 and 1.1 ml/min, respectively.

Determination of Partition Coefficient of KF Partition coefficient (*P*) for KF was evaluated to investigate it as an index of lipophilicity of KF around the pH in the gastroin-

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testinal tract. Five milliliters of buffer solution with various pH values containing KF ($2\text{ }\mu\text{g/ml}$) was added to an equal volume of ethyl acetate which had previously been saturated with each buffer solution. After shaking (60 strokes/min for 2 h at $18\pm 1\text{ }^{\circ}\text{C}$) to achieve equilibrium, the drug concentration in the buffer solution was analyzed by HPLC method. The buffer system used was $0.2\text{ M Na}_2\text{HPO}_4$ – 0.1 M citric acid (pH 3.0–10.0). The apparent partition coefficient (P') was calculated as the ratio of the equilibrium concentration of the organic phase to that of the aqueous phase. Partition coefficient (P) was obtained by the P' –pH profile.

Preparation of KF Suppository After the suppository base (Witepsol) had been melted at $45\pm 5\text{ }^{\circ}\text{C}$, KF powder was suspended in the base. The liquid was poured into a suppository mold for children (No. 3, Ikemoto Scientific Technology Co., Ltd., Tokyo). The mean weight of the suppositories was 0.66 g and each suppository contained 3.3 mg of KF. The suppositories were individually wrapped in aluminum foil and kept in the refrigerator until use.

Release of KF from the Suppositories An apparatus (model TMS-13, Toyama Sangyo Co., Osaka) for the measurement of KF release from the suppositories, was used according to the procedure reported by Muranishi *et al.*¹⁷⁾ The temperature of the water bath was maintained at $37\pm 0.1\text{ }^{\circ}\text{C}$. A phosphate buffer solution ($0.1\text{ M KH}_2\text{PO}_4$ – $0.1\text{ M Na}_2\text{HPO}_4$, 3 ml , pH 7.4) was employed as a release medium. A Millipore filter SSWP 04700 (Nihon Millipore Kogyo Co., Ltd., Tokyo), pore size $3\text{ }\mu\text{m}$, was employed as an artificial membrane. The rotation rate of the steel rod (3.4 cm i.d.) was 25 rpm . The receptor solution at pH 7.4 (300 ml) was stirred by a magnetic stirring bar at 100 rpm . Following dissolution of the suppository into the release medium, 0.5 ml of receptor solution was withdrawn at appropriate intervals. After each sampling, an equal volume of new medium ($37\text{ }^{\circ}\text{C}$) was added to the receptor solution.

In Vivo Experiments in Rabbits The KF solution (3.0 mg/ml) for intravenous and intranasal administration was prepared by adding KF powder to Zaditen[®] nasal solution. For intravenous administration, KF solution was injected into the ear vein. In the intranasal administration, KF solution (0.9 – 1.1 ml) was dropped into the nasal cavity of the rabbits by micropipet. For oral administration, KF powder diluted to 10% with lactose was placed in JP No. 4 and No. 2 capsules (Kobayashi Capsule Manufacturing, Himeji, Japan) to make doses of 1 and 5 mg/kg , respectively. For rectal administration, the size of the suppository was reduced by cutting down the original suppository according to the weight of each rabbit. The suppository was inserted into the rectum to a depth of about 3 cm from the anus; then the anus was closed with adhesive tape to prevent leakage. The doses of KF were 1 mg/kg body weight for intravenous, intranasal and rectal administration and 1 and 5 mg/kg body weight for oral administration. At least 1 week was allowed for the wash-out of the drug following the experiment. One milliliter of blood was withdrawn from the ear vein of each rabbit at appropriate time intervals after administration *via* these routes. The blood was heparinized immediately and centrifuged at 3000 rpm for 10 min. The plasma samples were stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Determination of KF in Plasma Plasma (0.4 ml) was added to 1.0 ml of acetonitrile containing internal standard

solution (50 ng/ml of ethyl *p*-aminobenzoate) and centrifuged at 3000 rpm for 10 min. The supernatant was evaporated *in vacuo* at $30\text{ }^{\circ}\text{C}$ and the residue was dissolved in $400\text{ }\mu\text{l}$ of 50% acetonitrile. After the solution was passed through a membrane filter (pore size $0.45\text{ }\mu\text{m}$, Millex[®]-LH, Nihon Millipore Kogyo Co., Ltd.), an aliquot (50 – $80\text{ }\mu\text{l}$) of this solution was injected onto the HPLC system described above.

Pharmacokinetic Analysis The pharmacokinetic parameters after intravenous administration were estimated by ordinary non-linear least squares (OLS) using MULTI (algorithm: Gauss–Newton method). The plasma data after intranasal, rectal or oral administration were analyzed by a two-compartment open model with first-order absorption by simultaneously fitting with the plasma data of intravenous administration. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained from the KF concentration–time curves, and the area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule. The AUC_{iv} value obtained by MULTI was almost the same as that obtained by the trapezoidal rule (MULTI, $516\pm 43.5\text{ ng}\cdot\text{h/ml}$; trapezoidal, $514\pm 48.2\text{ ng}\cdot\text{h/ml}$). The mean residence time (MRT) and the mean absorption time (MAT) were calculated as follows:

$$MRT = AUMC/AUC$$

$$MAT = MRT_{\text{intranasal, rectal or oral}} - MRT_{\text{iv}}$$

where $AUMC$ is the area under the first moment curve.

Statistical analysis was performed using Student's *t*-test with $p < 0.05$ as the minimal level of significance.

RESULTS

HPLC Analysis of KF in Plasma Figure 1 shows the chromatograms of blank plasma and plasma containing KF using a C18 column and a C8 column. When the C18 column was used, it was difficult to separate the peak of KF and the peak (14.0 min) derived from the blank plasma (KF, 13.1 min ; i.s., 25.1 min). When the C8 column was used, the peaks derived from the blank plasma did not interfere with the analysis of KF. We studied the effects of the concentration of 10, 50 and 100 mM of potassium bi-phosphate in the mobile phase and found that the peaks of KF and i.s. were made clear and sharp by the addition of 50 or 100 mM of potassium bi-phosphate. Ten millimolars of potassium bi-phosphate did not show a measurable effect in the analysis. By adjusting pH of the mobile phase to 4.0 with phosphoric acid, the retention times of KF and i.s. were shortened, and were 13.0 and 18.7 min , respectively.

Figure 2 shows the chromatograms of KF in plasma after its intravenous and oral administration in rabbits. The peaks of KF and i.s. were found in the chromatograms, however, the peak height of KF was found to be smaller after oral administration compared to that after intravenous administration.

Figure 3 shows the calibration curve of KF in plasma. Good linear correlation ($r=0.998$) was obtained between peak height ratios and KF concentrations (0, and 5 – 1000 ng/ml). The detection limit for KF in plasma was 5 ng/ml . The coefficients of variation for intra-day for KF at 10 and 1000 ng/ml were 2.7 and 1.4% ($n=15$), respectively. The coefficients of variation for inter-day at the same con-

centration described above were 4.2 and 1.5% ($n=15$), respectively.

Partition Coefficient of KF between Ethyl Acetate and Buffer Solution

Figure 4 shows the apparent partition co-

efficients (P') of KF between ethyl acetate and buffer solution in various pH values. Above pH 8 where KF exists in an unionized form, the partition coefficient (P) was estimated as 30.5 ($\log P=1.48$).

Release of KF from the Suppository Figure 5 shows the release percentage of KF from the suppository; this percentage increased gradually. The suppository melted within several minutes, but the release percentage of KF was less than 50% even after 3 h.

Plasma Concentrations of KF after Intravenous, Intranasal, Oral, and Rectal Administration in Rabbits After intravenous administration of KF, the plasma concentration-time curve showed two typical phases (distribution (α) phase and terminal (β) phase). From the plots, α and β were obtained. The curve fitted a two-compartment open model as shown in Fig. 6. Also, the plasma concentration-time curves after intranasal, oral and rectal administration fitted the two-compartment open model with first-order absorption (Fig. 6). The pharmacokinetic parameters and bioavailability parameters are summarized in Tables 1 and 2, respectively.

After intranasal administration, the plasma level of KF

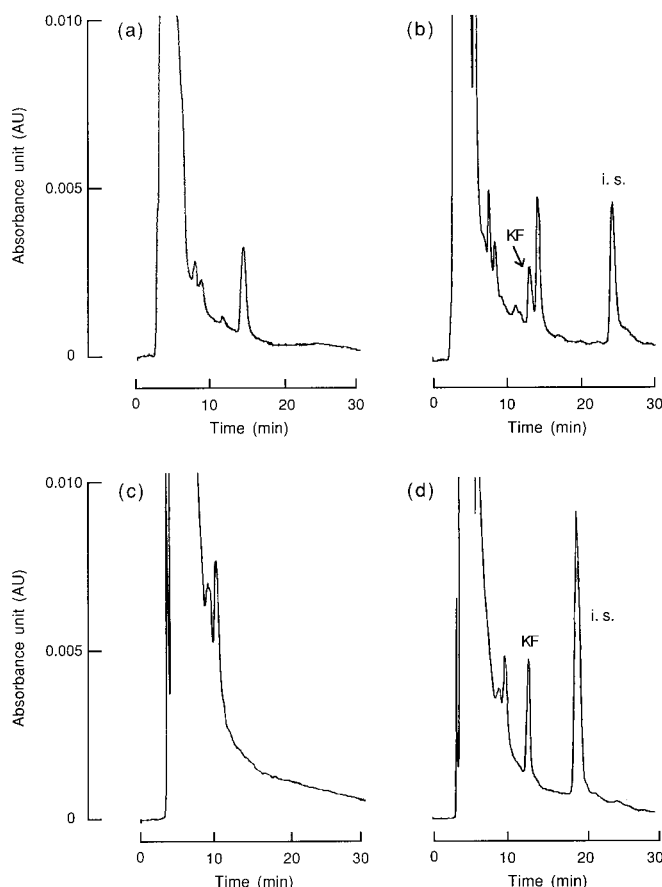


Fig. 1. HPLC Chromatograms of Blank Plasma and KF in Plasma Using C18 and C8 Columns

(a) Chromatograms in blank plasma using C18 column; (b) chromatograms of 100 ng/ml of KF and i.s. in plasma using C18 column; (c) chromatograms of blank plasma using C8 column; (d) chromatograms of 100 ng/ml of KF and i.s. in plasma using C8 column. Except for the column, HPLC conditions were the same as described in Materials and Methods.

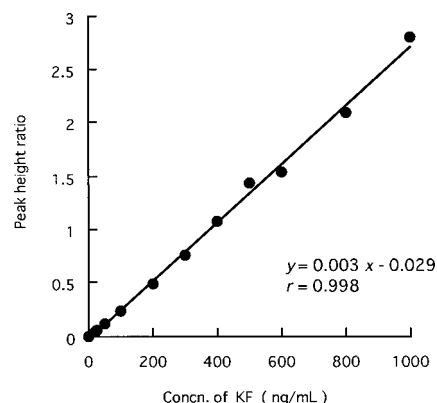


Fig. 3. Calibration Curve for KF in Plasma

The ratio of KF to internal standard was plotted against KF concentration from 10 to 1000 ng/ml. The linearity of the graph indicated a good correlation. Correlation factor (r) was 0.998 ($n=12$).

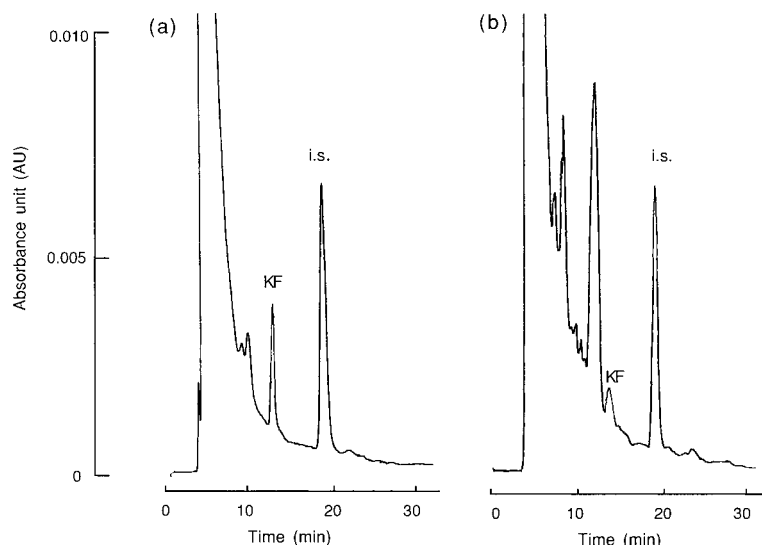


Fig. 2. HPLC Chromatograms of KF in Plasma after Intravenous (a) and Oral (b) Administration in Rabbit

(a) Plasma after intravenous administration (1 h); (b) plasma after oral administration (1.5 h).

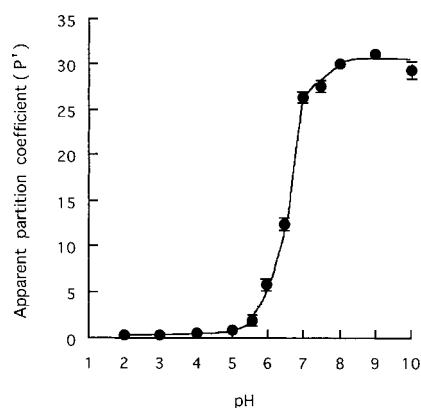


Fig. 4. Apparent Partition Coefficient-pH Profile of KF between Ethyl Acetate and Buffer Solutions

Each point represents the mean \pm S.E. ($n=3$).

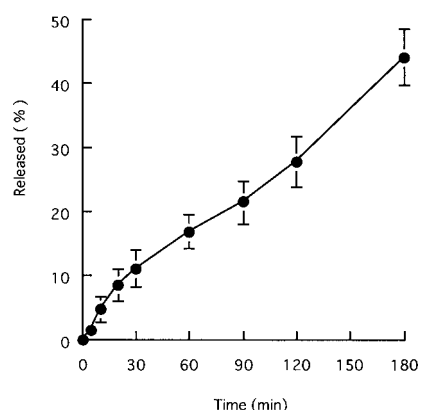


Fig. 5. Release Profile of KF from Suppositories into Buffer Solution (pH 7.4) at 37°C

Each point represents the mean \pm S.E. ($n=5$).

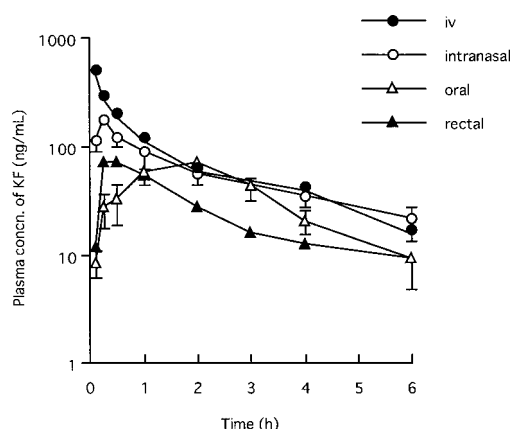


Fig. 6. Plasma Concentration of KF after Administration in Rabbits

Each point for intravenous (●), intranasal (○), oral (△) and rectal (▲) administration represents the mean \pm S.E. ($n=5-7$). The doses of KF were 1 mg/kg body weight for intravenous, intranasal and rectal administration, and 5 mg/kg body weight for oral administration.

rapidly reached a peak at 0.33 h (C_{\max} , 180 ng/ml), and after 2 h, the plasma concentrations were almost parallel to those after intravenous administration. The mean values of AUC after intravenous and intranasal administration were 514 and 338 ng·h/ml, respectively. The absolute bioavailability (F) of

Table 1. Pharmacokinetic Parameters after Administration of KF in Rabbits

Parameter	Intravenous ($n=7$)	Intranasal ($n=5$)	Rectal ($n=7$)	Oral ^{a)} ($n=7$)
k_{12} (h^{-1})	2.03 ± 0.60	2.28 ± 0.58	2.10 ± 0.60	2.29 ± 0.38
k_{21} (h^{-1})	1.18 ± 0.18	1.24 ± 0.17	1.08 ± 0.22	1.33 ± 0.07
k_e (h^{-1})	1.25 ± 0.16	1.16 ± 0.17	1.31 ± 0.16	1.27 ± 0.10
CL (l/h·kg)	2.04 ± 0.23	1.65 ± 0.05	1.99 ± 0.17	1.84 ± 0.13
V_1 (l/kg)	1.87 ± 0.38	1.52 ± 0.32	1.76 ± 0.30	1.21 ± 0.13
V_2 (l/kg)	2.75 ± 0.87	2.41 ± 0.04	3.03 ± 0.81	2.45 ± 0.44
V_{ss} (l/kg)	4.62 ± 1.06	3.98 ± 0.29	4.79 ± 0.99	3.66 ± 0.56
k_a (h^{-1})	—	6.24 ± 2.12^b	2.45 ± 0.47^b	0.63 ± 0.47

a) Oral data was calculated by conversion of a dose of 5 mg/kg into a 1 mg/kg dose equivalent. b) Significantly different from oral ($p < 0.05$). Each value represents the mean \pm S.E. ($n=5-7$).

Table 2. Bioavailability Parameters after Administration of KF in Rabbits

Parameter	Intravenous ($n=7$)	Intranasal ($n=5$)	Rectal ($n=7$)	Oral ^{a)} ($n=7$)
C_{\max} (ng/ml)	—	180 ± 22.5	68.9 ± 6.41	15.8 ± 2.00
T_{\max} (h)	—	0.35 ± 0.05^b	0.44 ± 0.09^b	1.51 ± 0.25
MRT (h)	1.46 ± 0.10^b	1.89 ± 0.16	1.77 ± 0.17^b	2.67 ± 0.28
AUC (ng·h/ml)	514 ± 48.2	338 ± 69.7	178 ± 16.6	44.2 ± 7.45
F (%)	—	65.8 ± 7.77	34.0 ± 3.46	8.32 ± 1.60
MAT (h)	—	0.54 ± 0.12	0.40 ± 0.14^b	1.24 ± 0.35

a) Oral data was calculated by conversion of a dose of 5 mg/kg into a 1 mg/kg dose equivalent. b) Significantly different from oral ($p < 0.05$). Each value represents the mean \pm S.E. ($n=5-7$).

KF after intranasal administration was 66%.

When a dose of 1 mg/kg KF was administered orally, most of the plasma concentrations of KF were below the detection limit of the HPLC assay. The results of a dose of 5 mg/kg administered orally are shown in Fig. 6. The mean value of AUC was 221 ng·h/ml. If the amount of absorption after oral administration of KF was proportional to the dose, when the dose was 1 mg/kg the value of AUC might be calculated as 44.2 ng·h/ml. The F after oral administration should be calculated as only 8%. The mean values of MRT and MAT were 2.67 and 1.24 h, respectively.

On the other hand, KF was absorbed rapidly after rectal administration, with the mean values of T_{\max} and the F 0.44 h and 34%, respectively. The mean value of F after rectal administration was 4.5 times larger than that obtained after oral administration. The values of MRT and MAT were significantly smaller than those after oral administration while the mean rate of absorption (k_a) was significantly larger than that of oral administration.

DISCUSSION

The C18 column has been conventionally used in HPLC analysis for KF.^{14,15} In this study, we found that the C8 column showed a good separation of the peak of KF and the peak derived from the blank plasma. Good reproducibility was obtained for KF analysis in plasma.

The partition coefficient of KF between ethyl acetate and buffer solution was 30.5 (Fig. 4). The value of P' is defined by the following equation:

$$P' = \frac{P}{1 + 10^{pK_a - pH}} \quad (1)$$

According to Eq. (1), the pK_a value of KF was calculated as 6.64. This value was very close to that ($pK_a=6.73$) reported by Lee *et al.*¹⁵⁾ The P' value ($P'=15$) around the pK_a of KF was large enough for the absorption of KF from the rectum or nasal cavity. The P' value below pH 4 might be very small for the absorption of KF in stomach, however, it was enough for the absorption of KF from the gastrointestinal tract. In fact, the absorption of KF was rapid after intranasal or rectal administration (Fig. 6).

Absolute bioavailability after oral administration was only 8% in this study. Grant *et al.*⁴⁾ and Grahnen *et al.*¹⁰⁾ reported that the bioavailability of KF was more than 50% after oral administration in human subjects. However, these analyses were employed by the RIA method. The metabolites of KF have been found as KF-*N*-glucuronide, *nor*-ketotifen and 10-OH ketotifen.^{4,12)} We doubt the possibility of measuring the total concentrations of unmetabolized KF and its metabolites by the RIA method. In such cases, the concentration of KF might be higher than the actual concentration. We found a large peak at 11.9 min in the HPLC chromatogram (Fig. 2b) after oral administration, but this peak was not detected in the chromatogram after intravenous, intranasal or rectal administration.

Unmetabolized KF was not excreted in urine.⁴⁾ Assuming the elimination route of KF is limited to metabolism, the following consideration may be made. In rabbits, the hepatic blood flow (Q_h) in the literature¹⁸⁾ is 10.2 l/h/2.5 kg. From the mean value of the total clearance (CL) = 2.04 l/h/kg, the hepatic extraction ratio (E_h) could be estimated as 0.50. Assuming the fraction of absorbed KF in the intestinal lumen (F_a) and the bioavailability in the small intestine (F_g) were 100%, the bioavailability of KF in the liver (F_h) would be 50%. However, the ratio of AUC_{oral} to AUC_{rectal} was 25%. The principal reason for the low bioavailability after oral administration might be attributable to the first-pass effect in the liver. The first-pass effect in the gastrointestinal tract might also be possible.

The total volume of distribution (V_{ss}) after intravenous administration was 4.6 l/kg. This result suggested that the distribution of KF into tissue was larger than into blood.

During the intranasal administration study, the rabbits showed drowsiness, which is known to be a typical adverse effect of KF.⁴⁾ According to a report on Novartis Pharma, this adverse effect was explained by the absorption of KF partially entered the gastrointestinal tract after intranasal administration. However, our study showed that the bioavailability of KF was less after oral administration than that intranasal administration. Drowsiness might be caused directly by the KF absorbed from the nasal cavity.

Chiang *et al.* reported the pharmacokinetics of the percutaneous absorption of KF in rabbits.¹⁹⁾ Plasma concentration of KF increased with addition of lauric acid. They found the

absolute bioavailability to be 50%. From our study, intranasal administration should be the most effective route for the absorption of KF.

The T_{max} after rectal administration of KF was found later than that after intranasal administration, although the values of MRT and MAT were smaller than those after intranasal or oral administration. The possible reason for this might be a slow release of KF from the suppository base. In *in vitro* experiments, the suppository melted within several minutes, but the release of KF might be dependent on the rate of its passing through the membrane filter of the apparatus.

The much less bioavailability of KF after oral administration might cause the variable effect or adverse effect in KF therapy. In terms of bioavailability, rectal administration of KF might be superior to oral administration. Further studies on the suppository base or a rectal absorption enhancer might allow KF therapy at a smaller dose than used for an oral dose.

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