Pharmacokinetics of an Antiallergic Agent, 1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1H-1, 4-diazepin-1-yl)-1H-benimidazole Difumarate (KG-2413) after Oral Administration: Interspecies Differences in Rats, Guinea Pigs and Dogs

Takanori SAKAI, Tsukasa HAMADA, Norio AWATA* and Jun WATANABE**

Pharmaceuticals Research Center, Kanebo, Ltd.,* 5-90 Tomobuchi-cho 1-chome, Miyakojima-ku, Osaka 534, Japan and Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Nagoya City University,** 3-1, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

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The pharmacokinetics of an antiallergic agent, 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1H-1, 4-diazepin-1-yl)-1H-benimidazole difumarate (KG-2413) after oral administration were studied in rats, guinea pigs and dogs. Maximum plasma level ($C_{\text{max}}$) appeared at 23, 32 and 51 min after dosing in rats, guinea pigs and dogs, respectively. Plasma half-lives of the terminal phase were comparable to those after intravenous administration in each animal species. Pronounced interspecies differences were observed in the $C_{\text{max}}$/dose and area under the plasma concentration-time curve ($AUC$/dose, namely, these values were high in guinea pigs and low in rats and dogs. The extents of bioavailability were 0.036 in rats (20 mg/kg), 0.50 in guinea pigs (2 mg/kg) and 0.052 in dogs (2 mg/kg). These variations in the pharmacokinetic parameters in the animals were assumed to be mostly due to the species difference in the hepatic intrinsic clearance.

Keywords — 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1H-1, 4-diazepin-1-yl)-1H-benimidazole difumarate (KG-2413); antiallergic agent; oral administration; pharmacokinetics; bioavailability; interspecies difference; rat; guinea pig; dog

Introduction

A new antiallergic agent, 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1H-1, 4-diazepin-1-yl)-1H-benimidazole difumarate (KG-2413) has developed as a preparation for oral administration.1)

We have previously reported the absorption and excretion of $^{14}$C-labelled KG-2413 in rats and guinea pigs.2) An experiment on the biliary excretion in rats showed that the orally administered radioactivity was almost completely excreted into the bile (88.5% of the dose) and urine (10.9% of the dose) by 24 h. Thus it appeared that KG-2413 was well absorbed from the intestinal tract of experimental animals. The elimination half-life ($t_{1/2}$) and the area under the plasma concentration-time curve ($AUC$/dose of total $^{14}$C after intravenous administration were comparable between rats and guinea pigs, while, after oral administration, the maximum plasma concentration ($C_{\text{max}}$/dose of total $^{14}$C in guinea pigs was about four times higher than that in rats.

In the pharmacokinetic study of unchanged KG-2413 base after intravenous administration,3) the volume of distribution in rats was comparable to that in dogs, and was about three times greater than that in guinea pigs. This might be due to the interspecies variations of unbound fractions of KG-2413 base in plasma ($f_u$), that is, the values of $f_u$ in rats, dogs and guinea pigs were 0.607, 0.603 and 0.189, respectively. It was suggested that KG-2413 might be more easily metabolized in rats than in guinea pigs, because the ratio of $AUC$/dose of unchanged KG-2413 base to $AUC$/dose of total $^{14}$C in rats was smaller than that in guinea pigs after intravenous administration.

Urinary excretion of unchanged KG-2413 base were negligible (less than 4% of the dose) in rats,4) guinea pigs4) and dogs,5) and biliary excretion of unchanged KG-2413 base was not detectable in rats.4) Therefore, KG-2413 base might be mainly eliminated by metabolism in animals.

Comparable values of total body clearance ($CL_{\text{tb}}$) and hepatic blood flow rate ($Q$) indicated that the hepatic intrinsic clearance ($CL_{\text{in}}$) was fairly larger than $Q$, and extent of bioavaila-
bility \( (F) \) was expected to be less than 0.5 in rats and guinea pigs.\(^6\) However, we were unable to estimate the accurate values of \( F \) and \( CL_{\text{int}} \) by the data in intravenous administration study alone.

In our previous papers which dealt with the method for quantitative determination of unchanged KG-2413 base in biological fluid, plasma concentration-time data of unchanged KG-2413 base after oral administration have been reported in dogs\(^6\) and guinea pigs,\(^7\) but systematic evaluations of species differences in pharmacokinetics of unchanged KG-2413 base after oral administration have not been done.

The purposes of this study were to clarify the interspecies variation in pharmacokinetics of unchanged KG-2413 base after oral administration and to elucidate the mechanism. In particular, we noted the bioavailability, eliminating capacity and rate of absorption. For these purposes, we performed the measurements of plasma concentrations of unchanged KG-2413 base after oral administration and estimations of various pharmacokinetic parameters in rats, guinea pigs and dogs.

**Experimental**

**Materials** — KG-2413 (Fig. 1) was identical to that used in the previous paper.\(^3\) Benzene for pesticide analysis and the other chemicals of special grade were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan.

**Animal Experiment** — Male Wistar rats weighing 220—250 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan), male Hartley guinea pigs weighing 260—370 g (Keali Co., Ltd., Osaka, Japan) and male beagle dogs weighing 9—13 kg (EDM Japan, Inc., Tokyo, Japan or White Eagle Laboratories, Inc. Doylestown, PA, U.S.A.) were used. Animals were fasted overnight prior to dosing. Rats and guinea pigs were given orally solutions of KG-2413 in saline by stomach tube at doses of 20 and 2 mg/kg (11.3 and 1.13 mg/kg as free base), respectively. Dogs were given orally aqueous solutions of KG-2413 being filled into No. 1 gelatin hard capsules as described in detail previously,\(^6\) at doses of 2, 10 and 15 mg/kg (1.13, 5.66 and 8.49 mg/kg as free base). Since the assay of the drug requires 1 ml of plasma sample, about 3 ml of blood was obtained from the inferior vena cava of each rat and guinea pig under ether anesthesia at a fixed time after administration. Right after the blood sampling, the animals were sacrificed. In dogs, the 3 ml blood samples as in the rats and guinea pigs were serially collected from the cephalic vein. One ml of plasma was prepared by centrifugation of the blood sample at 3000 rpm for 10 min. The concentrations of unchanged KG-2413 base in plasma were determined by using capillary gas chromatography with a nitrogen sensitive detector as described in detail previously.\(^6\) The detection limit was about 1 ng (as free base)/ml.

**Pharmacokinetic Analysis** — The KG-2413 base concentration (\( C_t \)) to time \( (t) \) data were fitted to the following equation using a nonlinear least-squares analysis program MULTI,\(^8\) based on the damping Gauss-Newton method,

\[
C_t = \left( \frac{F \cdot \text{dose}}{V_d} \right) \cdot \left\{ \frac{k_a'(k_a - k_e)}{e^{-k_e(t-t_{\text{lag}})} - e^{-k_a(t-t_{\text{lag}})}} \right\}
\]

(1)

where \( F, V_d, k_a, k_e \) and \( t_{\text{lag}} \) are extent of bioavailability, volume of distribution, rate constant of absorption, rate constant of elimination and lag time prior to the start of absorption, respectively. The mean plasma levels at each time point and a weight value of \( 1/(C_0) \) or \( 1/(C^2) \) were used for the least-squares regression analysis. The values of \( V_d/F, k_e, k_a \) and \( t_{\text{lag}} \) were expressed as an estimated value ± standard deviation (S.D.). Pharmacokinetic parameters were determined from \( V_d/F, k_e, k_a \) and \( t_{\text{lag}} \) using conventional equations, and the S.D. of each constant was calculated according to the "law of propagation of errors." The peak time \( (T_{\text{max}}) \), peak level \( (C_{\text{max}}) \), elimination half-life \( (t_{1/2}) \) and \( AUC \) were derived from Eqs. 2, 3, 4 and 5.

![Fig. 1. Chemical Structure of KG-2413](image)
respectively.

\[ T_{\text{max}} = \frac{1}{(k_a - k_e)} \cdot \ln \left( \frac{k_a}{k_e} \right) + t_{\text{lag}} \]  \hspace{1cm} (2)

\[ C_{\text{max}} = \frac{(F \cdot \text{dose} / V_d) \cdot \{k_a / (k_a - k_e)\} \cdot \left( e^{-k_e (T_{\text{max}} - t_{\text{lag}})} - e^{-k_e (T_{\text{max}} - t_{\text{lag}})} \} }{k_e} \]  \hspace{1cm} (3)

\[ t_{1/2} = 0.693/k_e \]  \hspace{1cm} (4)

\[ AUC = (F \cdot \text{dose}) / (V_d \cdot k_e) \]  \hspace{1cm} (5)

\( AUC \) was also calculated by the trapezoidal rule for the mean plasma levels and then extrapolated to infinity. \( F \) was calculated by a following equation,

\[ F = \frac{(AUC_{\text{po}}/\text{dose}_{\text{po}})}{(AUC_{\text{iv}}/\text{dose}_{\text{iv}})} \]  \hspace{1cm} (6)

where subscripts \( \text{po} \) and \( \text{iv} \) represent the oral and intravenous administration, respectively, and the previously reported values of \( AUC \) for intravenous administration are used.\(^3\) The \( AUC \) values after intravenous administration were 218, 421 and 369 ng·h·ml in rats, guinea pigs and dogs, respectively, at a dose of 2 mg/kg. At this dose, the pharmacokinetics after intravenous administration in rats, guinea pigs and dogs was considered to be linear.\(^3\) Blood clearance after oral administration \( (CL_{\text{oral}}) \) was estimated by a following equation,

\[ CL_{\text{oral}} = \frac{\text{dose}_{\text{po}} / AUC_{\text{po}} \cdot R_B}{R_B} \]  \hspace{1cm} (7)

where \( R_B \) represents the blood-to-plasma concentration ratio. Hepatic intrinsic clearance \( (CL_{\text{int}}) \) was calculated by Eq. 8.\(^9\) In this calculation, we assumed the complete absorption, the liver being the only eliminating organ and the "well-stirred" model.

\[ CL_{\text{oral}} = f_B \cdot CL_{\text{int}} \]  \hspace{1cm} (8)

where \( f_B \) represents the unbound fraction in blood and is estimated by a following equation,

\[ f_B = f_u / R_B \]  \hspace{1cm} (9)

where \( f_u \) is unbound fraction in plasma. We used the previously reported \( R_B \) and \( f_u \) values for KG-2413 base in rats, guinea pigs and dogs.\(^3\) The \( R_B \) values are 1.19, 0.70 and 1.14, and \( f_u \) values are 0.607, 0.189 and 0.603 in rats, guinea pigs and dogs, respectively. In each animal, \( R_B \) value was constant over the blood concentration range from 4 to 500 ng/ml.\(^3\) The calculation by using the plasma protein binding parameters of KG-2413 base\(^10\) indicated that the \( f_u \) values were also constant up to the plasma concentration of 200 and 200 ng/ml in rats and guinea pigs, respectively. In dogs, there is no data on the concentration dependency of plasma protein binding. But it was assumed that \( f_u \) values were also constant over the plasma concentration range in this pharmacokinetic study, because, judging from the values of \( f_u \) and \( R_B \), the characteristic of distribution in the blood in dogs resembled that in rats. The \( t \)-test was used for statistical comparisons between parameters;\(^11\) the level of significance chosen was 0.05.

**Results**

**Plasma Concentration in Rats, Guinea Pigs and Dogs after Oral Administration**

Figure 2 shows the plasma concentration-time curves of unchanged KG-2413 base in rats, guinea pigs and dogs after oral administration at doses of 20, 2 and 2 mg/kg, respectively. The pharmacokinetic parameters for these data are listed in Table I. There were no interspecies differences in the values of \( k_a \) among three animal species. The values of \( T_{\text{max}} \) were 23 min in rats, 32 min in guinea pigs and 51 min in dogs. The \( t_{1/2} \) in dogs was longer than that in rats or guinea pigs, and these values were comparable to the values after intravenous administration in each animal species. That is, the values of \( t_{1/2} \) were 1.1, 0.7 and 1.9 h in rats, guinea pigs and dogs, respectively, after intravenous administration.\(^3\) Pronounced interspecies differences were observed in the \( C_{\text{max}}/\text{dose} \) and \( AUC/\text{dose} \). the \( C_{\text{max}}/\text{dose} \) varied from 2.4 in dogs to 74 in guinea pigs, and \( AUC/\text{dose} \) varied from 4 in rats to 104 in guinea pigs. The \( AUC \) values estimated by the Eq. 5 were comparable with the values calculated by the trapezoidal rule (Table I). Therefore, the values of
Fig. 2. Plasma Concentration-Time Curves of Unchanged KG-2413 Base after Oral Administration to Rats (a), Guinea Pigs (b) and Dogs (c).

Each point represents the mean ± S.D. of three animals. Administered doses were 20 mg/kg in rats and 2 mg/kg in guinea pigs and dogs. The lines represent the least-squares fit of the data.

### Table 1. Pharmacokinetic Parameters of Unchanged KG-2413 Base Following Oral Administration to Rats, Guinea Pigs and Dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat (20 mg/kg, n=8)</th>
<th>Guinea Pig (2 mg/kg, n=7)</th>
<th>Dog (2 mg/kg, n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vd/F (l/kg)</td>
<td>146 ± 30</td>
<td>541 ± 0.30 k)</td>
<td>185 ± 0.17 l)</td>
</tr>
<tr>
<td>k_{a} (h^{-1})</td>
<td>7.65 ± 3.21</td>
<td>6.44 ± 1.33</td>
<td>3.42 ± 2.64</td>
</tr>
<tr>
<td>k_{e} (h^{-1})</td>
<td>0.98 ± 0.154</td>
<td>1.00 ± 0.06</td>
<td>0.32 ± 0.028 k)</td>
</tr>
<tr>
<td>t_{lag} (h)</td>
<td>0.0798 ± 0.0013</td>
<td>0.200 ± 0.010 k)</td>
<td>0.0862 ± 0.3106</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>0.388 ± 0.145</td>
<td>0.542 ± 0.061</td>
<td>0.850 ± 0.322</td>
</tr>
<tr>
<td>C_{max} (ng/ml)</td>
<td>57.3 ± 12.2</td>
<td>148 ± 9</td>
<td>4.78 ± 0.45</td>
</tr>
<tr>
<td>C_{max/dose} (ng/ml/[mg/kg])</td>
<td>2.87 ± 0.61</td>
<td>74.2 ± 4.3 k)</td>
<td>2.39 ± 0.23 l)</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>0.706 ± 0.111</td>
<td>0.691 ± 0.039</td>
<td>2.16 ± 0.19 k)</td>
</tr>
<tr>
<td>AUC_{v} (ng·h/ml)</td>
<td>79.0 ± 20.6</td>
<td>208 ± 16</td>
<td>19.1 ± 2.4</td>
</tr>
<tr>
<td>AUC_{dose} (ng·h/ml/[mg/kg])</td>
<td>3.95 ± 1.03</td>
<td>104 ± 8 k)</td>
<td>9.53 ± 1.20 k)</td>
</tr>
<tr>
<td>AUC_{v} (ng·h/ml)</td>
<td>86.0</td>
<td>214</td>
<td>19.1</td>
</tr>
<tr>
<td>AUC_{dose} (ng·h/ml/[mg/kg])</td>
<td>4.30</td>
<td>107</td>
<td>9.57</td>
</tr>
<tr>
<td>F (—)</td>
<td>0.0363 ± 0.0094</td>
<td>0.495 ± 0.039 k)</td>
<td>0.0516 ± 0.0065</td>
</tr>
<tr>
<td>CL_{oral} (ml/min/kg)</td>
<td>2010 ± 520</td>
<td>129 ± 10 k)</td>
<td>869 ± 110 l)</td>
</tr>
<tr>
<td>f_{B} (—)</td>
<td>0.51</td>
<td>0.27</td>
<td>0.53</td>
</tr>
<tr>
<td>CL_{int} (ml/min/kg)</td>
<td>3930 ± 1030</td>
<td>479 ± 38 k)</td>
<td>1640 ± 210 l)</td>
</tr>
</tbody>
</table>

Each value represents the computer-estimated parameter ± S.D. a) The number of data points used for the least-squares regression analysis. The mean plasma levels at each time point were used for the least-squares regression analysis. b) T_{max} value estimated by the Eq. 2 (see text). c) C_{max} value estimated by the Eq. 3 (see text). d) t_{1/2} value estimated by the Eq. 4 (see text). e) AUC value estimated by the Eq. 5 (see text). f) AUC value calculated by the trapezoidal rule. g) F value estimated by the Eq. 6 (see text). h) CL_{oral} value estimated by the Eq. 7 (see text). i) f_{B} value estimated by the Eq. 9 (see text). j) CL_{int} value estimated by the Eq. 8 (see text). k) p < 0.05 compared to rats. l) p < 0.05 compared to guinea pigs.
$AUC$ estimated from Eq. 5 were used for further analysis.

The values of $F$ and $CL_{oral}$ were calculated according to Eqs. 6 and 7, respectively, and are also listed in Table I. The values of $F$ were 0.036 in rats, 0.495 in guinea pigs and 0.052 in dogs. The value of $CL_{oral}$ in guinea pigs was significantly lower than that in rats or dogs.

**Dose Dependency in Dogs**

The dose dependency was examined in dogs in order to determine whether capacity-limited disposition occurs at higher doses. The plasma concentration of unchanged KG-2413 base after oral administration of 2 (the same data as in Fig. 2), 5 (the same data as in a previous report\(^6\)), 10 and 15 mg/kg to dogs are shown in Fig. 3. The plots of dose vs. $C_{max}$ and $AUC$ are represented in Fig. 4. The $C_{max}$ and $AUC$ showed non-linearity at the dose range of 2 to 15 mg/kg. The saturable disposition might exist in dogs after oral administration.

We also tried to examine the dose dependency in rats. If the linearity is held in rats, the $C_{max}$ should be nearly 6 ng/ml after oral administration at a dose of 2 mg/kg, because the $C_{max}$ was about 60 ng/ml at a dose of 20 mg/kg (Fig. 2, Table I). However, all plasma concentrations of unchanged KG-2413 base were less than the detection limit (1 ng/ml) after oral administration at a dose of 2 mg/kg in rats. Therefore, the behavior of KG-2413 base seemed to be also saturable in rats after oral administration.

$F$ can be obtained from the values of $AUC_{iv}$ and $AUC_{po}$ by Eq. 6 only in the linear condition. In the non-linear condition, when the dose dependent disposition of KG-2413 base was observed, the calculated $F$ represents the apparent $F$. Therefore, the $CL_{int}$ estimated by Eq. 8 also represents the apparent $CL_{int}$.

**Discussion**

Pharmacokinetics of an unchanged KG-2413 base after oral administration were studied in rats, guinea pigs and dogs. There were pronounced interspecies differences in $C_{max}$/dose and $AUC$/dose, which showed more than a 10-fold variation among three animal species.

![Fig. 3. Plasma Concentration–Time Curves of Unchanged KG-2413 Base after Oral Administration to Dogs at Doses of 2 (●), 5 (■), 10 (▲) and 15 (○) mg/kg](image1)

Each point represents the mean ± S.D. of three to eight animals. The lines represent the least-squares fit of the data.

![Fig. 4. Relationships between Dose and $C_{max}$ or $AUC$ after Oral Administration of KG-2413 in Dogs](image2)

Each point represents the computer-estimated value.
Pharmacokinetics of KG-2413 in Animals

We considered three possible explanations for the observed phenomena. They were the species differences in the extent of absorption, volume of distribution and first pass effect. Since KG-2413 seemed to be nearly completely absorbed from the intestinal tract of animals, it was impossible to explain the differences on the basis of the species differences in the extent of absorption. In addition, it was also difficult to elucidate the differences from the standpoint of species variation of the distribution volume, because the values of $Vd'_{B}$, which were estimated by the data after intravenous administration, showed at most a 3-fold variation among the three animal species i.e., from 2.66 l/kg in guinea pigs to 8.21 l/kg in dogs.

On the other hand, the values of $F$ showed a 13-fold variation between 0.036 in rats and 0.495 in guinea pigs (Table 1). This result suggested that the species difference existed in the extent of the first pass effect, because KG-2413 is well absorbed as mentioned above. Consequently, it is considered that the great species differences in $C_{\text{max}}$/dose and $AUC$/dose were mainly due to the species difference in the first pass effect.

Subsequently, the following analysis was done in order to clarify the source of the species difference of $F$. In the Eq. 6, $F$ is represented as a function of $AUC$ after oral and intravenous administration. On the other hand, $F$ is also expressed by three factors; hepatic blood flow rate ($Q$), unbound fraction in the blood ($f_{B}$) and hepatic intrinsic clearance ($CL_{\text{int}}$), as shown in Eq. 10.

$$F = Q/(Q + f_{B} \cdot CL_{\text{int}})$$  (10)

In this equation, we assumed complete absorption, the liver being the only eliminating organ and the “well-stirred” model. The values of $f_{B}$ calculated from Eq.9 and the values of $CL_{\text{int}}$ calculated from Eq. 8 are also listed in Table 1. The variation of $f_{B}$ was at most 2-times among three animal species. On the other hand, the values of $CL_{\text{int}}$ were 3900, 480 and 1600 ml/min/kg in rats, guinea pigs and dogs, respectively. Therefore, the great interspecies variation of $F$ might be mainly due to difference of $CL_{\text{int}}$, and partly due to difference of $f_{B}$. That is to say interspecies differences of pharmacokinetics observed after oral administration of KG-2413 might be mostly related to the ability of elimination in the liver.

Therefore, it seems interesting to investigate the hepatic transport, which is closely related to the ability of elimination in the liver, of KG-2413 base in experimental animals. There are three important factors which determine the behavior of a drug in the liver, i.e., influx, efflux and the sequestration (metabolism and/or excretion) process. We are now investigating the process of hepatic transport of KG-2413, which causes the species variation for rats and guinea pigs, by using an in vivo tissue sampling single injection technique.

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References


