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

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Interspecies differences in the pharmacokinetics of an antiallergic agent, 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1,4-diazepin-1-yl)benzimidazole difumarate (KG-2413) after intravenous administration were investigated in rats, guinea pigs and dogs. The disappearance of unchanged KG-2413 base was described by biexponential curves in all three animal species. The areas under the plasma concentration–time curves (AUC) in rats, guinea pigs and dogs were 218, 421 and 369 ng·h/ml, respectively, at a dose of 2 mg/kg. 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 difference in the 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. The first-order elimination rate constant from the central compartment (k_{el}) in dogs were smaller than those in rats and guinea pigs. Total body clearances (CL_{tot}) of KG-2413 base in rats and guinea pigs were comparable to the hepatic blood flow rate (Q) of each animal. Assuming that KG-2413 base is only eliminated in the liver, relatively rapid metabolism of KG-2413 base occurred in the liver of rats and guinea pigs. In dogs, extrahepatic elimination was suggested because the value of CL_{tot} seemed to be greater than that of Q.

Keywords 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1,4-diazepin-1-yl)benzimidazole difumarate (KG-2413); antiallergic agent; pharmacokinetics; interspecies difference; rat; guinea pig; dog; distribution volume; total body clearance

Introduction

1-(2-Ethoxyethyl)-2-(hexahydro-4-methyl-1,4-diazepin-1-yl)benzimidazole difumarate (KG-2413) has been developed as a new antiallergic agent.[1] In our previous pharmacokinetic study using ^{14}C-labelled KG-2413, there was no difference in the initial plasma concentration (C_0)/dose, elimination half-life ($t_{1/2}$) and area under the plasma concentration–time curve (AUC)/dose of total radioactivity between rats and guinea pigs after intravenous administration.[2] On the other hand, there were pronounced interspecies differences in the plasma protein binding of KG-2413 in rats and guinea pigs, that is, the binding affinity at the high affinity site in guinea pig plasma was sixteen times greater than that in rat plasma.[3] The plasma protein binding of a drug could occasionally affect the extent of the total body clearance (CL_{tot}), volume of distribution and $t_{1/2}$.

In our previous reports, which dealt with methods for the quantitative determination of unchanged KG-2413 base in plasma, the plasma concentration-time data in dogs[4] and guinea pigs[5] after oral administration were presented. In dogs, the C_{max} and $t_{1/2}$ were 24.1 ng/ml and *ca.* 2 h, respectively, at a dose of 5 mg/kg.[4] In guinea pigs, the C_{max} and $t_{1/2}$ were 90 ng/ml and *ca.* 1 h, respectively, at a dose of 2 mg/kg.[5] However, the detailed evaluation of species differences in distribution and elimination of unchanged KG-2413 base is impossible only with these pharmacokinetic data, *i.e.*, C_{max} and $t_{1/2}$.

In the present study, we investigated the pharmacokinetic behavior of unchanged KG-2413 base after intravenous administration in rats, guinea pigs and dogs, and compared these results with those of a previous study using ^{14}C-labelled KG-2413.[2] The second aim of this study was to find a relation between the pharmacokinetic properties of these animal species and their plasma protein bindings.

Experimental

Materials KG-2413 (Fig. 1) was synthesized and supplied by Fuji Chemical Industry, Co., Ltd., Toyama, Japan. ^{14}C-labelled KG-2413 was identical to that used previously.[2] All other chemicals of the highest grade were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan.

Animal Experiment Male Wistar rats weighing 190—230 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan), male Hartley guinea pigs weighing 230—310 g (Keali Co., Ltd., Osaka, Japan) and male beagle dogs weighing 9—10 kg (EDM Japan, Inc., Tokyo, Japan) were used. Animals were fasted overnight prior to dosing. KG-2413 was prepared as a solution in saline. Animals were given KG-2413 intravenously at a dose of 2 mg/kg (1.13 mg/kg as free base), and rats were also given doses of 10 and 20 mg/kg (5.66 and 11.3 mg/kg as free base, respectively). The solution of KG-2413 was injected into the femoral vein for rats (0.8 ml/kg) and guinea pigs (1.67 ml/kg) and into the cephalic vein for dogs (0.1 ml/kg). Since the assay of the drug requires an one ml of plasma sample, about 3 ml of blood sample 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 animal was sacrificed. In dogs, the same volume of blood samples as in the rats and guinea pigs were serially collected from the cephalic vein. One milliliter 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.[4] The detection limit was about 1 ng (as free base)/ml.

Plasma Protein Binding Plasma protein binding of KG-2413 in dog was evaluated by equilibrium dialysis according to the method described previously for rat and guinea pig plasma.[2]

Blood-to-Plasma Concentration Ratio (R_B) Since a method for the quantitative determination of KG-2413 base in whole blood has not been established, a conventional *in vitro* method was performed as follows. Blood was collected in heparinized tubes from each animal. One milliliter of the blood was preincubated for 5 min at 37 °C and the uptake was initiated by adding 10 μl of a stock KG-2413-^{14}C solution. The stock solution was prepared by dissolving KG-2413-^{14}C in phosphate-buffered isotonic saline (pH 7.4) at concentrations of 0.4, 4 and 50 μg (as free base)/ml. Preliminary experiments showed that the uptake of KG-2413 into blood cells was so rapid that a constant blood-to-plasma concentration ratio was obtained within 5 min. After incubation for 10 min at

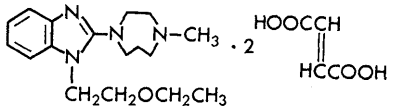

Fig. 1. Chemical Structure of KG-2413

37 °C, the blood and plasma concentrations of radioactivity were determined with a liquid scintillation spectrometer (model LSC-1050, Aloka Instruments Co., Tokyo, Japan) after combustion of samples with a Tricarb sample oxidizer (model 306B, Packard Instrument Co., Downers Grove, Ill., U.S.A.). The R_B value was calculated by dividing the blood concentration by the plasma concentration.

Pharmacokinetic Analysis The KG-2413 base concentration data were fitted to the equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ for the plasma concentration C_t at time t by non-linear least-squares regression using a weight value of $1/(C_t)^2$, and A, α, B and β were expressed as an estimated value ± standard deviation (S.D.).[6] All observed points (not the mean value) were used for the least-squares regression analysis. Pharmacokinetic constants were determined from the biexponential equation constants, *i.e.*, A, α, B and β, using conventional equations and the S.D. of each constant was calculated according to the "law of propagation of errors." The values of AUC, distribution volume of the central compartment (V_1), apparent volume of distribution (Vd'_β), first-order elimination rate constant from the central compartment (k_{el}) and elimination half-life at the β-phase ($t_{1/2\beta}$) were calculated using $AUC = A/\alpha + B/\beta$, $V_1 = \text{dose}/(A+B)$, $Vd'_\beta = \text{dose}/(\beta \cdot AUC)$, $k_{el} = (A+B)/AUC$ and $t_{1/2\beta} = 0.693/\beta$, respectively, AUC was also calculated using the trapezoidal rule. The t-test was used for statistical comparisons between parameters[7]; the levels of significance and "tendency" chosen were 0.05 and 0.1, respectively.

Results and Discussion

Plasma Concentration in Rats, Guinea Pigs and Dogs Figure 2 shows the time courses of KG-2413 base concentration in the plasma of rats, guinea pigs and dogs after intravenous administration of 2 mg/kg of the drug. The disposition of KG-2413 base in rats, guinea pigs and dogs was described by a two-compartment model. The pharmacokinetic parameters were calculated and are listed in Table I. AUC values estimated by means of the equation, $A/\alpha + B/\beta$ were comparable with the values calculated by applying the trapezoidal rule. Therefore, the values of AUC estimated from A, α, B and β were used for further analysis. The estimated values of AUC were 218, 421 and 369 ng·h/ml in rats, guinea pigs and dogs, respectively, that is, the value of AUC in guinea pigs was close to that in dogs, and greater than that in rats. This relationship between rats and guinea pigs was different from the result of the pharmacokinetic study using ^{14}C-labelled KG-2413 (see the introduction).[2]

The AUC ratio was estimated according the following equation,

$$AUC \text{ ratio} = (AUC_u/\text{dose})/(AUC_t/\text{dose}) \quad (1)$$

where AUC_u and AUC_t represent the AUC of unchanged KG-2413 and total radioactivity, respectively. The values of AUC_t were taken from a previous report.[2] Calculated AUC ratios were 0.19 in rats and 0.35 in guinea pigs. This suggested that KG-2413 might be more easily metabolized in rats and/or that the volumes of distribution of metabolites formed might be different between the two species.

The dose dependency was examined in rats in order to determine whether capacity-limited distribution and/or elimination occurs at higher doses. The plasma disappearance of unchanged KG-2413 base after intravenous administration of 2 (the same data as in Fig. 2), 10 and 20 mg/kg to rats is shown in Fig. 3. The disappearances of KG-2413 base were described by biexponential curves in all cases. The pharmacokinetic constants calculated are listed in Table II. The values of AUC increased proportionally to the administered dose. The values of α and β at a dose of 10 mg/kg were comparable to those at a dose of 2 mg/kg

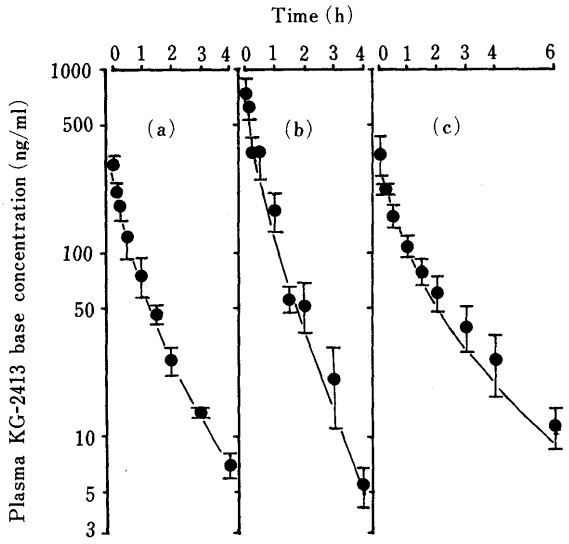

Fig. 2. Plasma Concentration–Time Curves of Unchanged KG-2413 Base after Intravenous Administration to Rats (a), Guinea Pigs (b) and Dogs (c) at a Dose of 2 mg/kg

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

TABLE I. Pharmacokinetic Parameters of Unchanged KG-2413 Base Following Intravenous Administration at a Dose of 2 mg/kg

	Rat ($n=27$)[a]	Guinea pig ($n=27$)[a]	Dog ($n=27$)[a]
A (ng/ml)	170 ± 32	482 ± 156[f]	224 ± 51
α (h^{-1})	2.21 ± 0.77	3.46 ± 2.02	1.53 ± 0.62
B (ng/ml)	92.6 ± 37.4	284 ± 102[f]	83.0 ± 48.9[h]
β (h^{-1})	0.655 ± 0.113	1.01 ± 0.12[g]	0.373 ± 0.116[f,i]
AUC[b] (ng·h/ml)	218 ± 30	421 ± 140	369 ± 80[f]
AUC_{tr}[c] (ng·h/ml)	238	521	419
V_1 (l/kg)	4.31 ± 0.41	1.47 ± 0.35[g]	3.68 ± 0.57[i]
Vd'_β (l/kg)	7.92 ± 1.75	2.66 ± 0.94[g]	8.21 ± 3.11[h]
k_{el} (h^{-1})	1.20 ± 0.12	1.82 ± 0.42	0.832 ± 0.126[g,i]
$t_{1/2\beta}$ (h)	1.06 ± 0.18	0.688 ± 0.079[f]	1.86 ± 0.58[h]
CL_{tot}[d] (ml/min/kg)	72.7 ± 10.0	64.0 ± 21.3	44.8 ± 9.7[f]
Q[e] (ml/min/kg)	64	45	24

Each value represents the computer-estimated parameter ± S.D. *a*) The number of data points used for the least-squares regression analysis. *b*) AUC value estimated by using the equation, $A/\alpha + B/\beta$. *c*) AUC value calculated by means of the trapezoidal rule. *d*) CL_{tot} value estimated by applying Eq. 2 (see text). *e*) Q values were obtained from reported values of blood or plasma flow rate, which are 16 ml blood/min in rats (250 g),[8] 40.3 ml blood/min in guinea pigs (896 g)[9] and 220 ml plasma/min in dogs (17 kg).[10] In dogs, plasma flow rate was converted to blood flow rate by using the hematocrit value (see text). *f*) $p<0.1$ compared to rats. *g*) $p<0.05$ compared to rats. *h*) $p<0.1$ compared to guinea pigs. *i*) $p<0.05$ compared to guinea pigs.

(Table I), and were lower than those at a dose of 20 mg/kg. These results indicated that the disposition process in rats was non-saturable up to 10 mg/kg. If the eliminating capacity of KG-2413 base is relatively large in guinea pigs and dogs as well as in rats, the pharmacokinetics in guinea pigs and dogs may be linear at a dose of 2 mg/kg.

Pharmacokinetic parameters in rats, guinea pigs and dogs for distribution (V_1, Vd'_β) and elimination (k_{el}, $t_{1/2\beta}$), derived from the values of A, B, α and β, are also listed in Table I. The volume of distribution (either V_1 or Vd'_β) in rats was close to that in dogs, and was about three times greater than that of guinea pigs. The first-order elimination rate constant from the central compartment (k_{el}) in dogs was smaller than that in rats and guinea pigs. Therefore,

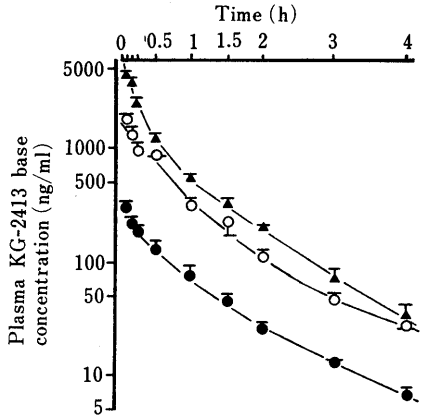

Fig. 3. Plasma Concentration–Time Curves of Unchanged KG-2413 Base after Intravenous Administration to Rats at Doses of 2 (●), 10 (○) and 20 (▲) mg/kg

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

TABLE II. Pharmacokinetic Parameters of Unchanged KG-2413 Base Following Intravenous Administration in Rats

	10 mg/kg ($n=27$)[a]	20 mg/kg ($n=27$)[a]
A (ng/ml)	1470±140	5200±660
α (h^{-1})	1.79±0.31	5.19±0.74[d,e]
B (ng/ml)	171±152	1280±140
β (h^{-1})	0.471±0.224	0.945±0.041[d,e]
AUC[b] (ng·h/ml)	1180±140	2350±340
AUC_{tr}[c] (ng·h/ml)	1290	2500

Each value represents the computer-estimated parameter±S.D. *a*) The number of data points used for the least-squares regression analysis. *b*) AUC value estimated by using the equation, $A/\alpha+B/\beta$. *c*) AUC value calculated by means of the trapezoidal rule. *d*) $p<0.05$ compared to the group of 2 mg/kg (Table I). *e*) $p<0.05$ compared to the group of 10 mg/kg.

interspecies difference in AUC (Table I) was due to differences in both the volume of distribution and k_{el}.

***In Vitro* Plasma Protein Binding** The unbound fraction of KG-2413 base (f_u) in dog plasma was 0.603±0.027 (mean±S.D., $n=3$) at a concentration of 60 ng/ml of KG-2413 base. Under the same conditions as in dogs, the value of f_u in rat and guinea pig plasma were 0.607 and 0.189, respectively.[2] The value of f_u in guinea pigs was about three times greater than that in rats and dogs. Therefore, the volume of distribution for KG-2413 base in rats, guinea pigs and dogs (Table I) seemed to be closely related to f_u in each animal.

***In Vitro* Blood-to-Plasma Concentration Ratio (R_B)** The R_B of KG-2413 in rats, guinea pigs and dogs is shown in Fig.4. In each animal, R_B was constant over the blood concentration range from 4 to 500 ng/ml. Similar mean values of R_B in both rats (1.19) and dogs (1.14) were observed, and were greater than those in guinea pigs (0.70). These observations suggested that KG-2413 base might be transferred to red blood cells more easily in rats and dogs compared with guinea pigs. This tendency was identical to the cases of the f_u and the volume of distribution.

Total Body Clearance (CL_{tot}) The CL_{tot} of KG-2413 base in rats, guinea pigs and dogs was estimated by applying following equation,

$$CL_{tot}=\text{dose}/(AUC\cdot R_B) \quad (2)$$

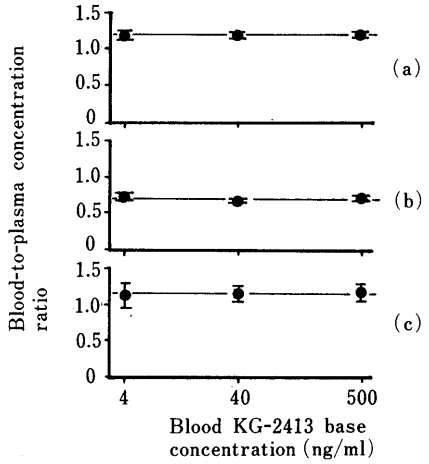

Fig. 4. Blood-to-Plasma Concentration Ratio of KG-2413 Base in Rats (a), Guinea Pigs (b) and Dogs (c) as a Function of the Total Blood Concentration

Each point represents the mean±S.D. of three animals.

Calculated CL_{tot} and hepatic blood flow rate (Q)[8-10] are also listed in Table I. The value of Q in dogs was estimated from hepatic plasma flow rate[10] by using the hematocrit value of dogs (0.45).[11] The values of CL_{tot} were comparable to those of Q in rats and guinea pigs. Assuming that KG-2413 base is eliminated only in the liver, which was supported by the study on metabolism in rats and guinea pigs,[12] and that the elimination of KG-2413 base in the liver is consistent with a "well-stirred" model, CL_{tot} is given by

$$CL_{tot}=Q\cdot f_B\cdot CL_{int}/(Q+f_B\cdot CL_{int}) \quad (3)$$

where f_B and CL_{int} represent the unbound fraction in blood and hepatic intrinsic clearance, respectively.[13] Therefore, a larger value of $f_B\cdot CL_{int}$ than Q might be responsible for the comparable values of both CL_{tot} and Q (Table I). In dogs, extrahepatic elimination was suggested because the value of CL_{tot} seemed to be slightly greater than that of Q.

KG-2413 has been developed as a preparation for oral administration. It was clarified that KG-2413 was nearly completely absorbed from the intestinal tract of rats and guinea pigs.[2] In the case of complete absorption, the liver being the only eliminating organ and the "well-stirred" model being assumed, the extent of bioavailability after oral administration (F) is represented by the following equation,[14]

$$F=Q/(Q+f_B\cdot CL_{int}) \quad (4)$$

Consequently, if the values of $f_B\cdot CL_{int}$ for KG-2413 base are greater than those of Q in rats and guinea pigs as mentioned above, the values of F in these animal species are less than 0.5.

In conclusion, the smaller volume of distribution in guinea pigs as compared with that in rats and dogs may be due to the smaller f_u, and extrahepatic elimination was suggested in dogs because the value of CL_{tot} seemed to be greater than that of Q. In addition, the rate of metabolism and/or the distribution volume of metabolites formed might vary among the animal species based on the comparison of AUC for unchanged KG-2413 base (AUC_u) and total radioactivity (AUC_t). However, in the present study,

we could only make a rough estimation of the values of F and CL_{int}. In general, the value of F is calculated from AUC after both oral and intravenous administrations. Under several assumptions, the clearance after oral administration (dose/AUC) can be represented as a function of f_B and CL_{int}.[14] Further study will be needed to clarify the pharmacokinetics of KG-2413 base after oral administration to experimental animals in order to evaluate the species variations of F and CL_{int} values.

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