The Biological Fate of 1-(2-Ethoxyethyl)-2-(4-methyl-1-homopiperazinyl)benzimidazole difumarate (KB-2413) I

Absorption and Excretion after Oral Administration to Rats and Guinea Pigs

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Summary

The absorption and excretion of 1-(2-ethoxyethyl)-2-(4-methyl-1-homopiperazinyl)benzimidazole difumarate (KB-2413) were studied in rats and guinea pigs after oral administration of KB-2413-¹⁴C. Maximum plasma levels (C_{max}) appeared at 0.25 and 0.5 h after dosing in rats and guinea pigs, respectively. In rats, both C_{max} and the area under the plasma concentration-time curve (AUC) increased proportionally to the administered dose up to 8mg/kg.

The radioactivity excreted into the urine and the AUC after oral dosing in rats and guinea pigs were close to those after intravenous dosing, and the orally administered radioactivity was almost completely excreted into the bile and urine in the bile-duct-cannulated rats. Thus, it was suggested that KB-2413 was nearly completely absorbed from the intestinal tract of rats and guinea pigs.

The percentages of the radioactivity excreted in feces were 65.3 and 58.9% of the dose within 96 h after oral administration of KB-2413-¹⁴C in rats and guinea pigs, respectively.

The radioactivity excreted into the bile was about 90% of the dose within 24 h after oral dosing in bile-duct-cannulated rats. The enterohepatic circulation in rats was suggested, since a considerable amount of the radioactivity was absorbed in the rat which was administered intraduodenally with the bile collected from other rats administered with KB-2413-¹⁴C orally.

1-(2-Ethoxyethyl)-2-(4-methyl-1-homopiperazinyl)benzimidazole difumarate (KB-2413) is a new benzimidazole derivative with strong antiallergic activity and less toxicity in animals compared with other antiallergics such as ketotifen and chlorpheniramine.^{1~4)}

This compound is readily dissolved in water and the pH of its aqueous solution is 3-4.

We have already reported about plasma levels of KB-2413 base after oral administration to dogs.⁵⁾ However, little information on the biological fate of KB-2413 has been still obtained. In the present study, the absorption and excretion after oral administration of ¹⁴C-labelled KB-2413 in rats and guinea pigs were investigated.

Experimental

Labelled compound—KB-2413-¹⁴C (Fig. 1) with the specific activity of 54.0 or 54.4 mCi/mmol was prepared in our laboratory and its radiochemical purity was more than 98 % in reverse isotope dilution analysis. The synthetic yield of this compound, starting from urea-¹⁴C, was 74 %.⁶⁾

Fig. 1. Chemical structure of KB-2413-¹⁴C.

* labelled with ¹⁴C.

Animals—Male Wistar rats weighing about 220 g (specific pathogen-free, Shizuoka Laboratory Animal Center) and male Hartley guinea pigs weighing about 300 g (Keali Co., Ltd.) were used, and fed with commercial pellet diets, CE-2 (Clea Japan, Inc.) and CG-3 (Clea Japan, Inc.), respectively. Environmental conditions were controlled at all times: relative humidity (55±5 %), temperature (22±0.5°C). Animals were fasted for 16 h prior to dosing and for 4 h post dosing. For the measurement of plasma levels, animals were chronically cannulated into the right external jugular vein with a silicone polymer tubing (i.d. 1.0 mm, o.d. 1.5 mm, Dow Corning) according to the method of Upton.⁷⁾

Administration of the drug—The labelled KB-2413 was diluted with the non-labelled KB-2413 and prepared as a solution in deionized water for oral administration or a solution in saline for intravenous administration. Rats were given orally or intravenously KB-2413 at a dose of 1 mg/kg, except for the experiments of the plasma levels measurement after oral administration (1, 4 and 8 mg/kg of KB-2413). All guinea pigs were given orally and intravenously at a dose of 2 mg/kg. All the doses as ¹⁴C were 50 μCi/kg, and all the volumes of administration were about 2.5 ml/kg. The solution of KB-2413-¹⁴C was given orally by stomach tube, or injected into the femoral vein.

Plasma level—After dosing, blood samples were collected at fixed times from the cannulae. Then 60-500 μl of plasma were prepared by centrifugation of the blood samples at 12,000 rpm for 5 min. The radioactivity in plasma was measured after its dilution with 0.5-1 ml of water.

Urinary, fecal and respiratory excretion—After dosing, each rat was kept separately in a metabolic cage (Metabolica CO-2, Sugiyama-gen Environmental Science Co., Ltd.).

Urine and feces were collected periodically for up to 96 h, separately, and the radioactive compounds in expired air were trapped by bubbling through an aqueous solution of 20 w/v % ethanolamine. In guinea pigs, another metabolic cage (CT-10 S, Clea Japan, Inc.) was used, and respiratory excretion was not measured. Urine and ethanolamine solutions obtained were made up to a constant volume with water, and the radioactivity in these solutions was measured. Feces were homogenized with about a double volume of water. An aliquot of the homogenate was combusted for radioactivity measurement.

Gastrointestinal absorption—After anesthetizing with ether, the rat stomach and intestine were exteriorized through a central mid-line incision and the bile duct was ligated. Then 4 loops were prepared from whole stomach and about 8 cm long of the duodenum, jejunum and ileum by ligation of the both ends. The solution of 10 μg (0.15 μCi in the radioactivity) of KB-2413- ^{14}C in 0.5 ml of water was injected into each loop with a syringe, and the abdominal wall was temporarily closed. Then 15, 30 and 60 min after the injection, the loop was removed separately and homogenized with about a double volume of water. An aliquot of the homogenate was combusted for radioactivity measurement. The amount of drug taken into the blood stream from the loop was estimated by substrating the residual amount in the lumen and gut wall from the administered dose.

Plasma protein binding—*In vitro* and *in vivo* experiments for protein binding of KB-2413 were carried out by equilibrium dialysis. *In vitro* experiments, 1 ml of rat or guinea pig plasma was put into a dialysis bag (#20/30, Visking Co.). Then the bag was suspended in a 35 $\times$ 100 mm test tube containing 5 ml of the solution of KB-2413- ^{14}C in 1/15 M phosphate buffer (pH 7.4, isotonic), and the test tube was agitated in a water bath at 37°C for 5 h. Preliminary dialysis indicated that the radioactivity reached equilibrium after 3–5 h incubation. After dialysis, the radioactivities of inner and outer solutions were determined, respectively, and the percent binding was calculated. The volume change in the inner phase after equilibrium was negligibly small. *In vivo* experiments, rat plasma samples obtained at 0.25, 2 and 6 h after oral administration of KB-2413- ^{14}C were used. One ml of the plasma sample was put into a dialysis bag. Then, the bag was suspended in a test tube containing 5 ml of 1/15 M phosphate buffer (pH 7.4, isotonic). The protein-bound radioactivity was determined as described above.

Biliary excretion—Cannulae (polyethylene tubing, i.d. 0.58 mm, o.d. 0.965 mm) were inserted into the bile ducts in ether-anesthetized rats. After complete recovery from anesthesia, KB-2413- ^{14}C was given orally. Then animals were fixed in each of Ballman cages (Natsume seisakusho Co., Ltd.) and the bile and urine were collected periodically for up to 48 and 24 h, respectively. The bile and urine samples obtained were made up to a constant volume with water, and the radioactivity was measured.

Enterohepatic circulation—KB-2413- ^{14}C was administered orally to the bile-duct-cannulated rats and the bile was collected for up to 6 h. One ml of the radioactive bile was administered into the duodenum of the other bile-duct-cannulated rats under ether anesthesia, and the abdominal wall was closed. Then the animals were fixed in each of Ballman cages (Natsume seisakusho Co., Ltd.) and the bile and urine were collected periodically. Feces were also collected for up to 24 h after dosing. The animals were sacrificed at 24 h after dosing, and their intestines with their contents were excised. The radioactivity

ties of the bile, urine and feces were measured as described above. The radioactivity of the intestine was measured in a similar manner as feces. The amount of the radioactivity taken into the systemic circulation from the intestinal tract was estimated by summing the amount of radioactivity excreted in urine and bile.

Determination of the radioactivity—The radioactivity of each sample was determined with Tri-carb liquid scintillation spectrometer (Model 2450 or 3385, Packard Instrument Co.) after dissolving directly in 12 ml of liquid scintillator (Scintisol® EX-H, Wako Pura Chemicals Ltd.) or combusting with a Tri-carb sample oxidizer (Model 306 or 306 B, Packard Instrument Co.). The quenching was corrected with an automatic external standard method.

Calculations—Concentrations of radioactivity in the plasma were expressed as ng equivalent of KB-2413 base per ml. The elimination half-life ($t_{1/2}$) of the plasma level was calculated by the least-squares regression analysis. Initial plasma level after intravenous administration (C_0) was estimated by back-extrapolation to zero time. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated by the trapezoidal rule for the observed values (0– t) and then extrapolated to infinity ($t-\infty$). All data were tested for statistically significant differences using Students' t -test in case of 2 groups, or F -test and Tukey's test in case of 3 groups.

Fig. 2. Plasma levels of the radioactivity following oral or intravenous administration of KB-2413- ^{14}C to rats. Each point represents the mean $\pm$ S.D.

—●: 8 mg/kg p.o. (n=3), ---●: 4 mg/kg p.o. (n=3),
—○: 1 mg/kg p.o. (n=3), ---○: 1 mg/kg i.v. (n=4)

Results

Plasma level

Figs. 2 and 3 show the plasma levels of the radioactivity after the administration of KB-2413- ^{14}C to rats and guinea pigs, respectively. Maximum plasma level appeared at 0.25 h after oral administration at doses of 1, 4 and 8 mg/kg in rats. The secondary peak was observed at 4-6 h after dosing. Thereafter, plasma level decreased with half-life of about 10 h at each dose.

Fig. 3. Plasma levels of the radioactivity following oral or intravenous administration of KB-2413- ^{14}C to guinea pigs at a dose of 2 mg/kg.

Each point represents the mean $\pm$ S.D.

—●—: Oral (n=4), —○—: Intravenous (n=3)

Plasma level after intravenous administration to rats decreased almost biexponentially with the half-life of about 6 h in terminal log linear phase, but it showed a small shoulder over 6-12 h after dosing.

Maximum plasma level appeared at 0.5 h after oral administration in guinea pigs, and the half-life of the blood plasma was about 6 h in the terminal phase. Plasma level after intravenous administration decreased biexponentially with half-life of about 8 h in terminal log linear phase.

Calculated pharmacokinetic parameters in rats and guinea pigs are summarized in Table I. In rats, both $C_{\max}$ and $\text{AUC}_{0-\infty}$ after oral administration increased proportionally to the administered doses. $C_{\max}$ in guinea pigs after oral dosing was about 4 times higher than that in rats at a normalized dose. However, there was no significant difference in

Table I Pharmacokinetic parameters of the radioactivity following oral or intravenous administration of KB-2413-¹⁴C to rats and guinea pigs.

Dose (mg/kg)	Route of administration	T _{max} (h)	C _{max} (ng/ml)	C ₀ (ng/ml)	t _{1/2(9-24h)} (h)	AUC _{0-∞} (ng·h/ml)
Rat						
1	p.o.(n=3)	0.25	46.3±3.6	—	8.6±3.5	432±86
4	p.o.(n=3)	0.25	251±33	—	12.8±3.6	1930±320
8	p.o.(n=3)	0.25	408±37	—	11.7±2.0	4090±1010
1	i.v.(n=4)	—	—	370±36	5.8±2.0	574±65
Guinea pig						
2	p.o.(n=4)	0.5	398±84	—	6.2±2.0	846±180
2	i.v.(n=3)	—	—	966±179	8.3±1.1	1200±210

Each value represents the mean±S.D.

Table II Cumulative urinary, fecal and expiratory excretion of the radioactivity following oral or intravenous administration of KB-2413-¹⁴C to rats and guinea pigs.

Biological sample	Time (h)	Recovery (% of dose±S.D.)			
		Rat		Guinea pig	
		p.o. (n=3)	i.v. (n=3)	p.o. (n=4)	i.v. (n=4)
Urine	0—6	12.4 ±2.8	17.7 ±7.7	—	—
	0—12	21.6 ±4.5	29.6 ±8.0	—	—
	0—24	29.5 ±5.2	37.5 ±4.5	37.3±3.7	45.3±13.3
	0—48	30.3 ±5.2	39.5 ±3.3	38.5±3.7	46.9±13.5
	0—72	30.5 ±5.2	39.9 ±3.5	38.9±3.7	47.7±13.8
	0—96	30.7 ±5.2	40.5 ±4.1	39.0±3.7	47.9±14.0
Feces	0—24	63.3 ±6.9	51.4 ±3.9	34.9±10.5	28.4±19.4
	0—48	65.0 ±7.7	58.4 ±6.8	51.8±4.7	42.4±18.4
	0—72	65.2 ±7.7	58.7 ±6.9	58.2±3.6	47.9±15.4
	0—96	65.3 ±7.7	58.9 ±7.0	58.9±3.6	50.0±14.2
Expired air	0—96	0.22±0.32	0.01±0.01	—	—
Total	0—96	96.2 ±3.7	99.4 ±5.8	97.9±1.0	97.8±2.8

— : not determined.

AUC_{0-∞} between rats and guinea pigs at the normalized dose.

Urinary, fecal and respiratory excretion

The cumulative urinary, fecal and respiratory excretion of the radioactivity after oral and intravenous administration of KB-2413-¹⁴C to rats and guinea pigs is shown in Table II. Urinary excretion of the radioactivity by 96 h after oral and intravenous administration were 30.7 and 40.5 % of the dose in rats, and were 39.0 and 47.9 % in guinea pigs, respectively. In rats, the percentages of the radioactivity excreted into the expired air after oral and intravenous administration were nearly zero.

Gastrointestinal absorption

The percent absorption of the radioactivity after injection of KB-2413-¹⁴C into the ligated stomach and loops of different parts of rat intestine is shown in Fig. 4. In 15 min after the administration, the percent absorption from the duodenum, jejunum and ileum were 56.0, 38.8 and 25.7 % of the dose, respectively. In 60 min, about 80 % of the dose was abso-

Fig. 4. Absorption of KB-2413-¹⁴C from rat gastrointestinal tracts.

Each point represents the mean $\pm$ S.D. of 3 animals.

—○ : Stomach, —● : Duodenum, —□ : Jejunum, —▲ : Ileum

rbled from each part of the small intestine. On the other hand, the radioactivity absorbed from stomach was about 1 % of the dose by 60 min.

Plasma protein binding

In vitro study, the percent binding of the radioactivity to rat plasma protein were 38.9 ± 3.3 (n=3), 38.6 ± 4.8 (n=3) and 39.3 ± 5.4 % (n=3) at concentrations of 6, 20 and 60 ng/ml, respectively, of KB-2413-¹⁴C base in outer solution. On the other hand, the percent binding of the radioactivity to guinea pig plasma protein was 81.1 ± 9.0 % (n=3) at a concentration of 60 ng/ml of KB-2413-¹⁴C base in outer solution.

In vivo study, the percent of the radioactivity bound to rat plasma protein were 23.1 ± 4.2 % (n=4) at 0.25 h, 34.8 ± 8.1 % (n=3) at 2 h and 19.5 ± 6.4 % (n=4) at 6 h after oral administration of KB-2413-¹⁴C.

Fig. 5. Cumulative biliary excretion of the radioactivity following oral administration of KB-2413-¹⁴C to rats at a dose of 1 mg/kg.

Each point represents the mean $\pm$ S.D. of 4 animals.

Biliary excretion

The cumulative biliary excretion of the radioactivity after oral administration of KB-2413- ^{14}C is shown in Fig. 5. The percent excretion of the radioactivity were 77.3, 88.5 and 89.6 % of the dose during 4, 24 and 48 h after dosing, respectively. Urinary excretion of the radioactivity determined simultaneously, though not shown in the figure, was 10.9 ± 4.0 % ($n=3$) of the dose during 24 h after administration.

Enterohepatic circulation

KB-2413- ^{14}C was administered to the bile-duct-cannulated rats, and bile was collected for up to 6 h. The radioactivity excreted into the bile was about 80 % of the dose. One ml ($2.08 \mu\text{Ci}$) of the radioactive bile was given into the duodenum of the other bile-duct-cannulated rats. The percent absorption of the radioactive materials in the bile were 9.3, 57.8 and 72.5 % of the administered radioactivity in the bile during 6, 12 and 24 h after dosing, respectively, as shown in Table III.

Table III Cumulative biliary, urinary and fecal excretion of the radioactivity following intraduodenal administration of the radioactive bile.

Biological sample	Time (h)	Recovery (% of dose $\pm$ S.D., $n=5$)
Bile	0—6	5.2 ± 2.6
	0—12	32.5 ± 8.6
	0—24	40.6 ± 5.9
Urine	0—6	4.0 ± 1.3
	0—12	25.3 ± 8.2
	0—24	32.0 ± 5.8
Bile+Urine	0—6	9.3 ± 1.8
	0—12	57.8 ± 14.9
	0—24	72.5 ± 6.4
Feces	0—24	5.5 ± 4.3
Intestine	0—24	22.0 ± 3.5
Total	0—24	100.0 ± 1.6

Discussion

Since the maximum plasma level appeared at 0.25 and 0.5 h after oral dosing in rats and guinea pigs, respectively (Figs. 2, 3), the intestinal absorption of KB-2413 in rats and guinea pigs seemed to be relatively fast. *In situ* absorption study in rats showed that KB-2413 was well absorbed from the small intestinal tract particularly from the duodenum (Fig. 4). This might be correlated to rapid absorption of KB-2413. The percentages of the radioactivity excreted into the urine within 96 h after oral administration and the AUC in rats and guinea pigs were close to those after intravenous administration (Table II, Table I). 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, Fig. 5) and urine (10.9 % of the dose) by 24 h. Thus it was concluded that KB-2413 was nearly completely absorbed from the intestinal tract of rats and guinea pigs.

The radioactivities excreted into the feces were more than one-half the orally and

intravenously administered KB-2413- ^{14}C in rats and guinea pigs (Table II). Experiments on fecal and urinary excretion after oral and intravenous dose of KB-2413- ^{14}C in rats and guinea pigs showed an almost complete excretion of the radioactivity from the body within 96 h (Table II).

The radioactivity excreted into the bile was about 90 % of the dose within 24 h after oral administration of KB-2413- ^{14}C in rats (Fig. 5). In guinea pigs, the percent of total radioactivity excreted into the feces and urine after oral dose were comparable to those in rats (Table II). These results suggested that the marked excretion of the radioactivity into the bile might have occurred also in guinea pigs. Study on the enterohepatic circulation in rats suggested that a considerable amount of the radioactivity excreted into the bile was reabsorbed from the intestinal tract.

There was no difference in C_0 , $t_{1/2}$ and AUC between rats and guinea pigs after intravenous administration at a normalized dose (Table I). After oral administration, however, the secondary plasma peak observed in rats was not found in guinea pigs. The C_{max} in rats was smaller than that in guinea pigs at a normalized dose (Table I). These differences between rats and guinea pigs might be due to the differences in the behavior of KB-2413- ^{14}C in the gut and/or liver. And this might be also correlated to the difference of plasma protein binding in rats and guinea pigs.

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