Pharmacokinetics of [6]-Gingerol after Intravenous Administration in Rats with Acute Renal or Hepatic Failure

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The pharmacokinetics of [6]-gingerol were investigated in rats with acute renal failure induced by bilateral nephrectomy, or those with acute hepatic failure induced by a single oral administration of carbon tetrachloride (CCl_4), to clarify the contribution of the kidney and liver to the elimination process of [6]-gingerol. After bolus intravenous administration, a plasma concentration–time curve of [6]-gingerol was illustrated by a two-compartment open model. There was no significant difference in either the plasma concentration–time curve or any pharmacokinetic parameters between the control and nephrectomized rats. It is suggested, therefore, that renal excretion does not contribute at all to the disappearance of [6]-gingerol from plasma in rats. In contrast, hepatic intoxication with CCl_4 elevated the plasma concentration of [6]-gingerol at the terminal phase. Its elimination half-life increased significantly, from 8.5 to 11.0 min, in CCl_4-intoxicated rats. The extent of [6]-gingerol bound to serum protein was more than 90% and was affected very slightly by the CCl_4-intoxication. These aspects indicate that [6]-gingerol is eliminated partly by the liver.

Keywords [6]-gingerol; ginger; *Zingiber officinale*; pharmacokinetics; bilateral nephrectomy; renal elimination; carbon tetrachloride-intoxication; hepatic elimination; rat

"Shokyo" is the rhizome of ginger, *Zingiber officinale* ROSCOE, and a useful crude drug in traditional Chinese herbal medicine.[1] It has been known that Shokyo has various pharmacological effects, *e.g.*, antipyretic, cardiotonic, anticonvulsive and analgesic effects, which have been reported to be predominantly based on [6]-gingerol (Fig. 1), one of the important pungent components of ginger.[2–7] Shokyo is prescribed in most Chinese medicines and, therefore, it is important to clarify the pharmacokinetic properties of its major efficacious constituent, [6]-gingerol.

In our previous study,[8] we investigated the pharmacokinetics of [6]-gingerol after bolus intravenous (i.v.) administration in rats. It was found that [6]-gingerol was cleared very rapidly from plasma in spite of relatively extensive binding of this compound to serum protein. However, no specific, major route of elimination has been clarified for this compound. In this paper, therefore, to clarify the contribution of renal and hepatic elimination to the total body clearance of [6]-gingerol, we examined the pharmacokinetics of this compound in bilaterally nephrectomized or carbon tetrachloride (CCl_4)-intoxicated rats.

Experimental

Materials [6]-Gingerol was obtained from the rhizome of ginger. The details for the methods of isolation and purification have been given in a previous report.[1] All other chemicals and solvents used were commercially available and of either analytical or liquid chromatographic grade.

Animals Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan), weighing 345 to 426 g (12 weeks old), were used. All rats were fasted with free access to water overnight (about 17 h) before the operation. Under light anesthesia with ether, rats were cannulated in the right external jugular vein with silicone tubing (i.d. 1.0 mm; o.d. 1.5 mm, Dow Corning, Tokyo, Japan) in accordance with the method reported previously.[9] These chronically-cannulated rats were used for all the following experiments.

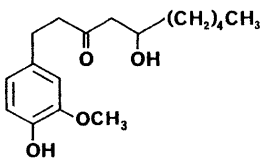

Fig. 1. Chemical Structure of [6]-Gingerol

[6]-Gingerol Solution [6]-Gingerol was quickly weighed and dissolved in acetonitrile. An aliquot of this solution was taken in a glass-tube and the solvent was completely evaporated under a gentle stream of nitrogen at 40 °C. The residue was uniformly dispersed in a 5% Tween 80/0.9% NaCl solution.

Pharmacokinetic Study in Nephrectomized Rats Acute renal failure was produced by bilateral nephrectomy.[10] The left and right renal arteries and veins were ligated immediately after the cannulation into the jugular vein. The sham-operation for the nephrectomy was performed in the control rats. After the operation, the rats were housed for about 1 h to relieve them of the anesthesia, then they were administered [6]-gingerol. Both rat groups (control and nephrectomized) were given a bolus i.v. dose of 3 mg/kg of [6]-gingerol *via* the cannula. The blood sample (about 330 μl) was collected from the cannula into heparinized micro-tubes at 2, 4, 7, 10, 15, 20 and 30 min after the injection. Plasma (150 μl) was immediately separated from the blood by centrifugation.

Pharmacokinetic Study in CCl_4-Intoxicated Rats The rats with acute hepatic failure were experimentally prepared by a single oral administration of CCl_4 in olive oil (1 : 1, v/v) at the dose of 1 ml/kg about 6 h after the cannulation. To the control rats, 0.5 ml/kg of olive oil was administered orally. Eighteen h after the treatment, the rats were administered a bolus i.v. dose of 1.5 mg/kg of [6]-gingerol *via* the cannula. Blood (450 μl) was withdrawn from the cannula at 2, 4, 7, 10, 15, 20, 25 and 30 min after the administration, and plasma (200 μl) was separated from the heparinized blood. In the experiment for determining the serum protein binding of [6]-gingerol in the control and CCl_4-intoxicated rats, about 2 to 3 ml of the blood was collected at about 2 to 4 min after the administration of [6]-gingerol and separated to serum. A portion of the serum was ultrafiltered using a micropartition system (MPS-3, Amicon Corp., Danvers, MA, U.S.A.). The extent of [6]-gingerol binding to serum protein was estimated from the concentrations in the serum and its filtrate.

Analytical Procedures [6]-Gingerol: Plasma concentrations of [6]-gingerol were determined by high-performance liquid chromatography. In the experiment for acute renal failure, a sample preparation including extraction, evaporation and reconstitution was performed in exactly the same way as in the previous paper.[8] In the nephrectomized rats, there was no interference of plasma endogenous constituents with the separation of the peak of [6]-gingerol. In the experiment for hepatic failure, some modifications were made. Briefly, 500 μl of acetonitrile was added to 200 μl of the plasma sample. The mixture was centrifuged and 600 μl of the supernatant was evaporated to dryness. The residue was reconstituted and injected into the liquid chromatograph equipped with a ultraviolet (UV) spectrophotometric detector SPD-10A (Shimadzu, Kyoto, Japan). Under these conditions, the assay limit of [6]-gingerol was 0.05 μg/ml as the plasma concentration. The coefficient of variation for the assay at this

concentration was 7.5% ($n=5$).

Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT): To validate the onset of hepatic dysfunction, the activities of GOT and GPT in the serum were measured for the control and CCl_4-treated rats at 18 h after the treatment. The activities of serum GOT and GPT were each determined with a commercial test kit, Unikitrate (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan).

Data Analysis All of the individual plasma concentration–time data after i.v. administration of [6]-gingerol was analyzed by a non-linear least squares regression program, MULTI.[11] A two-compartment open model was used to estimate the following pharmacokinetic parameters: total body clearance (CL_{tot}), elimination half-life ($t_{1/2\beta}$), area under the plasma concentration–time curve (AUC), distribution volumes of central compartment (V_1) and peripheral compartment (V_2), by conventional equations.

Statistics The Student's t-test was utilized to estimate a statistical significance of difference between the means of two groups. A p value of 0.05 or less was considered to be statistically significant.

Results

Pharmacokinetics of [6]-Gingerol in Nephrectomized Rats Plasma concentration–time curves of [6]-gingerol after the bolus i.v. dosing (3 mg/kg) to the control and bilaterally nephrectomized rats are shown in Fig. 2. The plasma level of [6]-gingerol appeared to decline biexponentially with time in both groups. The nephrectomized rats seemed to yield almost the same plasma concentration–time curve as that in the control rats. A two-compartment open model was most adequate to describe this data. Corresponding pharmacokinetic parameters estimated by analysis of the data are given in Table I. The estimates are expressed as the mean and standard deviation (S.D.) of the values obtained from individual rats. The elimination half-life at the terminal phase for the control rats was 9.33 ± 1.20 min and that of the nephrectomized rats was 11.0 ± 1.9 min. There was no significant difference between these estimates. The total body clearance was 47.8 ± 11.4 ml/min/kg and 52.7 ± 23.4 ml/min/kg for the control and nephrectomized rats, respectively. Essentially, no significant change was observed either in the plasma concentration data or in the pharmacokinetic parameters of the nephrectomized group from the control.

Pharmacokinetics of [6]-Gingerol in CCl_4-Intoxicated Rats Table II shows the activities of GOT and GPT in the control and CCl_4-intoxicated rats. The activities of these transaminases in the serum of the control rats were almost similar to the values reported for the untreated rats.[12] It can be clearly observed that the activities of these transaminases in serum increased remarkably in the CCl_4-intoxicated rats. The single oral treatment with CCl_4 increased the activities of GOT and GPT by about 40- and 50-fold, respectively, under the present experimental

Table I. Pharmacokinetic Parameters for [6]-Gingerol after Bolus Intravenous Administration (3 mg/kg) to Control and Bilaterally Nephrectomized Rats

Parameter		Control	Nephrectomized
A	(μg/ml)	42.1 ±33.2	49.9 ±58.4
B	(μg/ml)	2.23 ± 0.29	1.78 ± 0.61
α	(min^{-1})	1.05 ± 0.23	0.95 ± 0.33
β	($10^{-2}min^{-1}$)	7.52 ± 0.88	6.44 ± 1.15
$t_{1/2\beta}$	(min)	9.33 ± 1.20	11.0 ± 1.9
AUC	(μg·min/ml)	66.3 ±18.6	70.6 ±41.2
CL_{tot}	(ml/min/kg)	47.8 ±11.4	52.7 ±23.4
V_1	(1/kg)	0.0947± 0.0530	0.136± 0.117
V_2	(1/kg)	0.257 ± 0.114	0.330± 0.229

Plasma concentration (C_p)–time (t) curves of [6]-gingerol after i.v. administration were fitted to a bi-exponential equation, $C_p=Ae^{-\alpha t}+Be^{-\beta t}$, by program MULTI [Weight (i)$=1/C_p i$]. Results are expressed as the mean±S.D. of 6 rats. There is no significant difference between the two groups.

Table II. Biochemical Parameters in Serum of Control or CCl_4-Intoxicated Rats

	Control	CCl_4-intoxicated
n	4	5
GOT[a]	87.8±6.2	3352±859[b]
GPT[a]	23.5±1.6	1146±226[b]

Results are expressed as the mean±S.D. Serum was collected 18 h after the treatment. *a*) Karmen's unit. *b*) Significant difference from control rats at $p<0.001$.

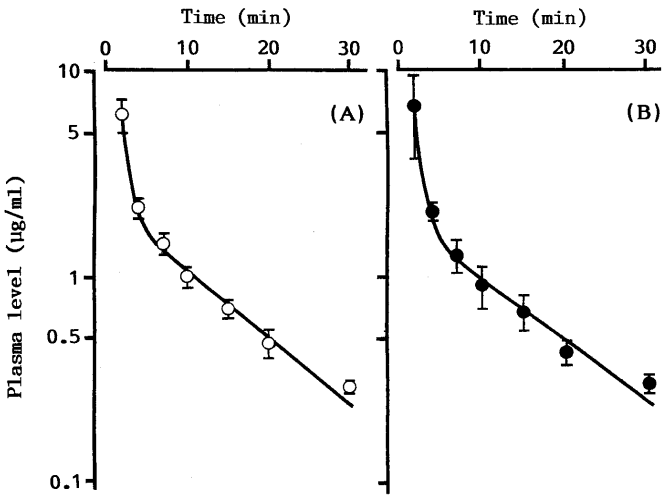

Fig. 2. Plasma Concentration–Time Profiles of [6]-Gingerol after Bolus Intravenous Administration (3 mg/kg) to (A) Control and (B) Bilaterally Nephrectomized Rats

Each point represents the mean and S.D. of 6 rats. The solid lines express the simulated curve for the mean data. There is no significant difference between the two groups.

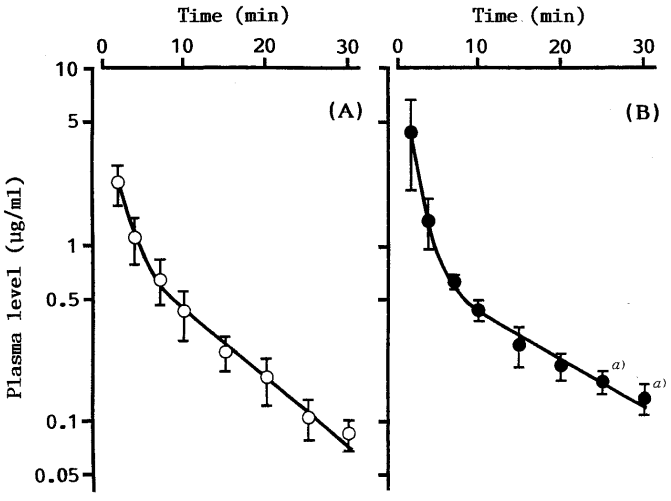

Fig. 3. Plasma Concentration–Time Profiles of [6]-Gingerol after Bolus Intravenous Administration (1.5 mg/kg) to (A) Control and (B) CCl_4-Intoxicated Rats

Each point represents the mean and S.D. of 4 rats. The solid lines express the simulated curve for the mean data. *a*) Significant difference from control rats at $p<0.05$.

TABLE III. Pharmacokinetic Parameters for [6]-Gingerol after Bolus Intravenous Administration (1.5 mg/kg) to Control and CCl_4-Intoxicated Rats

Parameter		Control	CCl_4-intoxicated
A	(μg/ml)	5.92 ± 3.85	19.4 ±15.4
B	(μg/ml)	0.927± 0.362	0.753± 0.084
α	(min^{-1})	0.638± 0.265	0.720± 0.236
β	($10^{-2}min^{-1}$)	8.30 ± 1.19	6.32 ± 0.25[a]
$t_{1/2\beta}$	(min)	8.51 ± 1.42	11.0 ± 0.5[a]
AUC	(μg·min/ml)	19.8 ± 5.4	35.9 ±16.6
CL_{tot}	(ml/min/kg)	80.7 ±23.5	50.1 ±24.3
V_1	(l/kg)	0.270± 0.120	0.152± 0.151
V_2	(l/kg)	0.341± 0.138	0.244± 0.169

Plasma concentration (C_p)–time (t) curves of [6]-gingerol after i.v. administration were fitted to a bi-exponential equation, $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, by program MULTI [Weight (i) = $1/C_p i$]. Results are expressed as the mean±S.D. of 4 rats. *a*) Significant difference from control rats at $p<0.05$.

TABLE IV. Serum Protein Binding of [6]-Gingerol after Bolus Intravenous Administration (1.5 mg/kg) to Control or CCl_4-Intoxicated Rats

Treatment	Serum concentration (μg/ml)		Bound fraction (%)
	Total	Unbound	
Control	2.44±0.82	0.180±0.035	92.2±2.3
CCl_4-intoxicated	4.74±2.23	0.535±0.336	89.3±1.9[a]

Serum was obtained from the rat at about 2 to 4 min after administration of [6]-gingerol. Results are expressed as the mean±S.D. of 6 rats. *a*) Significant difference from control rats at $p<0.05$.

condition.

Plasma concentration–time curves of [6]-gingerol after the bolus i.v. dosing (1.5 mg/kg) to the control and CCl_4-intoxicated rats are shown in Fig. 3. Similarly to the control rats, the plasma concentration of [6]-gingerol in the CCl_4-intoxicated rats declined in a biexponential fashion. However, the slope of the plasma concentration–time curve at the terminal phase tended to decrease following CCl_4-intoxication, and significantly higher plasma concentrations of [6]-gingerol compared with the control rats were observed in the CCl_4-intoxicated rats at 25 and 30 min after administration. The pharmacokinetic parameters of [6]-gingerol estimated from the plasma concentration–time data are listed in Table III. The elimination half-lives of [6]-gingerol at the terminal phase were 8.51±1.42 and 11.0±0.5 min for the control and CCl_4-intoxicated rats, respectively, and a significant difference was observed between both groups. Furthermore, the CCl_4-intoxication tended to reduce the total body clearance of this compound to about 62% of the control value.

The fractions of [6]-gingerol bound to serum protein after bolus i.v. administration to both the control and CCl_4-intoxicated rats are shown in Table IV. The bound fraction of this compound in the control rats was 92.2% and that in the CCl_4-intoxicated rats was 89.3%. Although a significant difference was observed between the two groups, the magnitude of change was relatively small.

Discussion

In our previous study,[8] it was found that [6]-gingerol is rapidly cleared from blood plasma after bolus i.v. administration in rats. [6]-Gingerol was eliminated with a short terminal half-life in spite of a relatively high extent of binding to serum protein (approximately 92%). The total body clearance was relatively large compared to creatinine clearance in rats. Therefore, it was suggested that [6]-gingerol had a specific route or mechanism of distribution and elimination. In the present paper, we investigated the contribution of kidney and liver, which are known to be predominant organs taking part in the disposition of xenobiotics to pharmacokinetics, especially the elimination process, of [6]-gingerol by using rats which were deficient in renal or hepatic function due to acute failure of the organs.

In the first place, the pharmacokinetics of [6]-gingerol in rats with acute renal failure induced by bilateral nephrectomy was compared with that in the control rats. There was neither a significant difference nor a consistent tendency in the plasma concentration–time profile and any pharmacokinetic parameters between the control and nephrectomized rats (Fig. 2, Table I). This result demonstrates that [6]-gingerol is not excreted into urine as either unchanged or metabolized. Therefore, it is suggested that renal elimination does not contribute at all to the plasma clearance of [6]-gingerol.

Due to the limitation of sampling time-points and a large variation of the plasma concentration in the initial distribution phase, there was a large standard deviation for several pharmacokinetic parameters. This was similar to previous results.[8] As a matter of interest, there was a considerable difference in the plasma concentration–time profiles, in both initial distribution and terminal elimination phases, between the previous rats and the present control against the nephrectomized rats in spite of the same dose of [6]-gingerol.[8] In fact, some pharmacokinetic parameters such as the elimination half-life and total body clearance were found to be larger in this work than in previous experiments. The exact reason for these discrepancies is uncertain, but they may be due to the complicated effects of anesthesia retained in the rats even 1 h after ether removal and surgical conditions.

The contribution of liver to the plasma clearance of [6]-gingerol was examined using rats with acute hepatic failure induced by a single oral administration of CCl_4.

CCl_4 is widely used to cause hepatic cellular injury to create a pathological model of hepatic disease. A single oral administration of CCl_4 can produce hepatic centrilobular necrosis,[13] leading to a delay in the plasma disappearance of the drug excreted from the liver.[14] As shown in Table II, the activity of the transaminases in the serum obtained from the CCl_4-intoxicated rats was about 40 or 50 times higher than the control rats. These results indicate that, in the present study, CCl_4-treatment caused serious hepatotoxicity, resulting in a reduction of hepatic function in rats.

The results shown in Fig. 3 and Table III indicate that elimination of [6]-gingerol from plasma was delayed by CCl_4-intoxication. It is well known that hepatic failure may decrease the content of albumin in the blood and change the protein binding which affects the distribution and elimination of some drugs. Therefore, the effect of CCl_4-intoxication on the the serum protein binding of [6]-gingerol was examined. From the results shown in Table IV, the change in the bound fraction of [6]-gingerol by

hepatic failure was very slight. Consequently, the increase in the elimination half-life of [6]-gingerol in the CCl_4-intoxicated rats may be not due to a change in protein binding, but to the reduction of the hepatic function itself, including metabolic activity and bile flow rate, induced by CCl_4.[12,14,15] These aspects indicate that the liver is one of the organs which takes part in the elimination of [6]-gingerol. In addition, since [6]-gingerol is only slightly excreted into bile (unpublished data), the effect of CCl_4 on the hepatic handling of this compound is considered to be relevant to metabolic activities in the liver.

In conclusion, it has been clarified that there is no contribution of renal clearance to the total body clearance of [6]-gingerol, but that [6]-gingerol is eliminated partly by the liver. However, the present findings do not suggest that only hepatic elimination contributes to the high plasma clearance of [6]-gingerol. Other organ participation in the relatively high clearance of [6]-gingerol, including extensive uptake and/or rapid transformation, should be considered.

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