Pharmacokinetics of [6]-Gingerol after Intravenous Administration in Rats

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A high-performance liquid chromatographic method to determine [6]-gingerol, a pungent constituent of ginger, in rat plasma was developed and a pharmacokinetic study was performed in rats. Quantitative analysis with high reproducibility was achieved for [6]-gingerol over the concentration range of 0.2–40 µg/mL. After bolus intravenous administration at a dose of 3 mg/kg, the plasma concentration-time curve was described by a two-compartment open model. [6]-Gingerol was rapidly cleared from plasma with a terminal half-life of 7.23 min and a total body clearance of 16.8 ml/min/kg. Serum protein binding of [6]-gingerol was 92.4%.

Keywords [6]-gingerol; pharmacokinetics; determination; intravenous administration; serum protein binding; rat; HPLC

The rhizome of ginger (Zingiber officinale Roscoe) has been used not only as a seasoning spice but also as a useful crude drug in Chinese medicine, named “Shokoyo.” It is considered to be used as antiemetic, antitussive, stomach tonic, carminative, stimulant and diuretic.1,2) [6]-Gingerol has been known to be an important pungent component of ginger.3,4) It has been found that [6]-gingerol possesses various pharmacological effects, for example, inhibition of spontaneous motor activity, antipyretic and analgesic effects,5) and cardiotoxic effect.6) However, little has been known about the pharmacokinetics of [6]-gingerol. The purpose of the present study is to develop a method for determination of [6]-gingerol in rat plasma with high-performance liquid chromatography (HPLC) and to clarify the pharmacokinetics of [6]-gingerol after bolus intravenous (i.v.) administration in rats.

Materials and Methods

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.7) All other reagents were commercial products of analytical grade.

Animals Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan), weighing 350–419 g, were used. Under light anesthesia with ether each rat was cannulated with silicone tubing in the right jugular vein and fasted overnight (about 24 h) before the experiments.

Drug Administration [6]-Gingerol was uniformly dispersed in normal saline containing 5% Tween 80. The rats were given a bolus i.v. dose of 3 mg/kg of [6]-gingerol via the cannula.

HPLC Assay

Extraction: To 150 µL of plasma sample, 500 µL of CH3CN was added, and vortexed for 20 s. The mixture was centrifuged at 1.5 × 10^4 rpm for 5 min at room temperature and the supernatant (550 µL) obtained was evaporated to dryness at 40°C under a gentle stream of N2 gas. The residue was reconstituted in 75 µL of CH3OH–H2O (4:6) and an aliquot (20 µL) was injected into the chromatograph.

Chromatography: A Shimazu Model LC-9A pump (Shimadzu, Kyoto, Japan), equipped with a Shimadzu Model SPD-6A variable-wavelength spectrophotometric detector, was used. Samples were chromatographed with an analytical column of Wakosil SC18 (4.6 mm i.d. × 150 mm) at 40°C. The mobile phase was CH3CN–H2O (45:55) with a flow-rate of 1.0 mL/min. The eluate was monitored by the spectrophotometric detector at 280 nm with a sensitivity of 0.005 a.u.f.s.

Calibration Graph: To blank plasma were added known amounts of [6]-gingerol in the final concentration range of 0.2–40 µg/mL. These plasma samples were treated according to the above determination procedure. The calibration curve was constructed by using the peak area of [6]-gingerol.

Reproducibility: Blood samples were obtained from the rats at appropriate times after the administration of [6]-gingerol. Aliquots (150 µL each) of the plasma samples were repeatedly analyzed according to the above procedure.

Accuracy: To the plasma samples which were obtained from the rat administered [6]-gingerol were added known amounts of [6]-gingerol and then this compound in the plasma was determined. The recovery of [6]-gingerol was calculated by comparing the experimental value with the corresponding theoretical value.

Pharmacokinetic Study [6]-Gingerol was administered to the rats and blood samples (about 330 µL) were withdrawn through the cannula into the heparinized tubes at 2, 4, 7, 10, 15, 20, 25, 30, 45 and 60 min after the administration. After centrifugation, the plasma was immediately separated and kept frozen until the analysis. Plasma concentration–time data after i.v. administration of [6]-gingerol was analyzed by a non-linear least squares regression program, MULTI.

Serum Protein Binding Study About 2.5 ml of blood was collected from the rats at about 2 to 4 min after the administration of [6]-gingerol. The serum was immediately obtained by centrifugation with a serum separator (Fibrichin, Takazono Sango Co., Ltd., Osaka, Japan) and was utilized for the ultrafiltration method by using a micropartition system MPS-3 (Amicon Corp., Danvers, MA, U.S.A.). Since the preliminary experiments indicated that 11.5% of the [6]-gingerol was adsorbed by this system, we corrected the drug concentration in the filtrate by using this value. The extent of binding of [6]-gingerol to serum protein was estimated by the drug concentrations in the serum and the filtrate.

Results

Figure 1 shows representative chromatograms for a plasma blank and plasma sample obtained from the rat which was given [6]-gingerol. The peak for [6]-gingerol was well separated from the peaks which seemed to be derived from endogenous materials in the rat plasma. The retention time for [6]-gingerol was about 8.8 min.

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TABLE I. Precision and Accuracy on the Determination of [6]-Gingerol (6-G) in Rat Plasma

<table>
<thead>
<tr>
<th>Reproducibility$^a$</th>
<th>Recovery$^b$</th>
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<tr>
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<td></td>
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<tr>
<td>6-G level (μg/ml)</td>
<td></td>
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<tr>
<td>Mean</td>
<td>35.8</td>
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<tr>
<td>S.D.</td>
<td>2.65</td>
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<tr>
<td>0.310</td>
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$^a$ Based on 10 determinations. $^b$ Based on 5 determinations.

Fig. 2. Plasma Concentration–Time Profile of [6]-Gingerol after Bolus Intravenous Administration (3 mg/kg) to Rats

Each point and vertical bar represent the mean and S.D. of 6 rats.

TABLE II. Pharmacokinetic Parameters for [6]-Gingerol after Bolus Intravenous Administration (3 mg/kg) to Rats

<table>
<thead>
<tr>
<th>Parameter$^a$</th>
<th>Mean ± S.D.$^b$</th>
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<tr>
<td>$A$ (μg/ml)</td>
<td>283.1 ± 197.8</td>
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<tr>
<td>$B$ (μg/ml)</td>
<td>3.44 ± 0.93</td>
</tr>
<tr>
<td>$k$ (min$^{-1}$)</td>
<td>1.30 ± 0.33</td>
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<tr>
<td>$\beta$ (10$^{-3}$ min$^{-1}$)</td>
<td>9.69 ± 1.05</td>
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<tr>
<td>$t_{1/2}$ (min)</td>
<td>7.23 ± 0.83</td>
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<tr>
<td>$AUC$ (μg·min/ml)</td>
<td>238.1 ± 114.0</td>
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<tr>
<td>$CL$ (ml/min/kg)</td>
<td>16.8 ± 10.9</td>
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<tr>
<td>$V_{p}$ (ml/kg)</td>
<td>20.3 ± 18.7</td>
</tr>
<tr>
<td>$V_{p}'$ (ml/kg)</td>
<td>36.8 ± 41.5</td>
</tr>
</tbody>
</table>

$^a$ Estimated by program MULTI [Weight(1)=1/Cl]. $^b$ Based on the data of 6 rats. $^c$ Distribution volume of central compartment. $^d$ Distribution volume of peripheral compartment.

Calibration line for [6]-gingerol was linear over the concentration range of 0.2—40 μg/ml. The regression equation was $y = 3484x - 642$, $r = 1.000$, where $y$ is the peak-area of the drug, $x$ is plasma concentration (μg/ml) of the drug and $r$ is the correlation coefficient. The values of coefficient of variation (C.V.) for the peak-area at [6]-gingerol concentrations tested were within 8.1%. Similar results were also obtained when serum was used in place of plasma.

Table I shows the precision and accuracy data on the determination of [6]-gingerol. The precision of the determination of [6]-gingerol in plasma was examined by performing ten replicate analyses at three different concentrations of the drug in plasma samples. The C.V. values ranged from 1.7 to 4.8%. The recovery data was obtained when drug was added at two different concentrations to the rat plasma sample. The average recovery at both concentrations was nearly 100%.

After the bolus i.v. administration of 3 mg/kg of [6]-gingerol, plasma level of the drug declined with time in a biexponential pattern as shown in Fig. 2. A two-compartment open model was found to describe the data most adequately. The corresponding pharmacokinetic parameters which were estimated by the analysis of the data obtained from the individual rat are given in Table II. The elimination half-life at the terminal phase was 7.23 min and the total body clearance was 16.8 ml/min/kg.

The results of serum protein binding experiments are shown in Fig. 3. In the serum concentration range of 5.24—40.9 μg/ml, the bound fraction of [6]-gingerol was almost constant and its mean value was 92.4%.

Discussion

In order to elucidate the clinical effects of Chinese medical preparations, it is important to investigate their pharmacokinetics. Ginger is prescribed in about 50% of Chinese medical prescriptions.$^7$ Thus, we selected [6]-gingerol, an effective component of ginger, as a model compound and investigated the pharmacokinetics of this compound in rats. In the first place, a sensitive and selective method for the determination of [6]-gingerol in biological fluids was required to investigate the pharmacokinetics of this compound. The determination methods of [6]-gingerol in the "Zingiberis Rhizoma" by using gas chromatography-mass spectrometry (GC-MS),$^3$ HPLC$^7$ and supercritical fluid chromatography (SFC)$^9$ have been reported. However, no paper concerning the method in the biological fluids such as blood, plasma or serum has been published. We have developed a simple and precise method for determination of [6]-gingerol in the rat plasma by HPLC. The pretreatment procedure in the present determination method consisted of only two processes, the deproteinization and concentration, without any solvent extraction. Therefore, nothing was employed as the internal standard. The data listed in Table I indicated that the present method had satisfactory precision and accuracy in spite of the absence of an internal standard.

Using the present determination method we have performed a pharmacokinetic study on [6]-gingerol after i.v. administration. Limitation of sampling time-points as well as a large variation of the plasma concentrations in the
initial phase might yield relatively large standard deviation for several pharmacokinetic parameters. It was not possible to monitor plasma levels of [6]-gingerol beyond 30 min since its concentrations were quite close to the limit of sensitivity. However, the plasma concentration of [6]-gingerol at 30 min was less than about 0.1% of that attained immediately after i.v. administration. Therefore, it is thought that the plasma concentration-time profile has reached an elimination phase at about 30 min. From the present kinetic analysis, it was found that [6]-gingerol was cleared very rapidly from plasma with a short terminal half-life in rats.

It has been reported that the pharmacological effect was maintained until 180 min after i.v. administration at a dose of 3.5 mg/kg. Thus for [6]-gingerol, there may be a small discrepancy or time-shift between the duration of pharmacological effect and the plasma level due to rapid clearance. The following possible explanations may be proposed for this: [6]-gingerol may be irreversibly sequestered in the tissues where it acts; [6]-gingerol may be effective at a much lower plasma concentration than those detected by the present assay; some active metabolites may be produced. It is also interesting that [6]-gingerol was cleared quickly from plasma in spite of the relatively high extent of binding to serum protein. From these aspects, it is possible that [6]-gingerol has a specific route or mechanism of distribution and elimination. Further detailed work is necessary to clarify the above discrepancy and specific disposition mechanism of [6]-gingerol and is now in progress.

References