Metabolism of [6]-gingerol in rats

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Abstract

The metabolic fate of [6]-gingerol, one of the active constituents of Zingiber officinale Roscoe, was investigated using rats. The bile of rats orally administered [6]-gingerol was shown to contain a major metabolite (1) by HPLC analysis. Although the metabolites derived from [6]-gingerol were not detected in the urine, the ethyl acetate extract of the urine after enzymatic hydrolysis was shown to contain six minor metabolites (2–7). Their structures were determined to be (S)-[6]-gingerol-4’-O-β-glucuronide (1), vanillic acid (2), ferulic acid (3), (S)-(+)-(4-hydroxy-6-oxo-8-(4-hydroxy-3-methoxypyphenyl) octanoic acid (4), 4-(4-hydroxy-3-methoxyphenyl)butanoic acid (5), 9-hydroxy [6]-gingerol (6) and (S)-(+)-[6]-gingerol (7) based on spectroscopic and chemical data. The total cumulative amount of 1 excreted in the bile and 2–7 in the urine during 60 h after the oral administration of [6]-gingerol were approximately 48% and 16% of the dose, respectively. The excretion of 2–7 in the urine decreased after gut sterilization. On the other hand, the incubations of [6]-gingerol with rat liver showed the presence of 9-hydroxy [6]-gingerol, gingerdiol (8), and (S)-[6]-gingerol-4’-O-β-glucuronide (1). These findings suggest that the gut flora and enzymes in the liver play an important part in the metabolism of [6]-gingerol. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: [6]-gingerol; metabolites; bile; urine

Introduction

The rhizome of ginger (Zingiber officinale Roscoe, Zingiberaceae) is widely used as a common condiment for a variety of foods and beverages. In addition to its extensive...
utilization as a spice, the fresh or processed rhizome is a useful crude drug in traditional Chinese medicine. It is considered to possess stomachic, carminative, stimulant, diuretic, bechic, and antiemetic properties [1, 2]. Chemical studies on the pungent principles of ginger have been carried out by a number of investigators, and S-(+)-[6]-gingerol as a major pungent substance has been isolated [3–10]. [6]-Gingerol has been found to possess a variety of interesting pharmacological effects, for example, analgesic, antipyretic, cardiotonic effects, and inhibition of spontaneous motor activities and prostaglandin biosynthesis [11–13]. In a pharmacokinetic study, Ding et al. reported the plasma concentration of [6]-gingerol after the bolus intravenous administration in rats [14]. Takahashi et al. showed biotransformation of [6]-gingerol by Aspergillus niger [15]. However, little information is available on the metabolic fate of [6]-gingerol in mammals, except for the in vitro metabolism using the rat hepatic postmitochondrial fraction [16]. The purpose of the present study, therefore, is to clarify the metabolic fate of [6]-gingerol in mammals. We report here the structure and cumulative excretion of biliary and urinary metabolites when [6]-gingerol is orally administered to rat.

Methods

Chemicals

(S)-(+-)[6]-Gingerol was isolated from Zingiber officinale Roscoe (Zingiberaceae). (S)-[6]-gingerol-4’-O-β-glucuronide (1), (S)-(+-)4-hydroxy-6-oxo-8-(4-hydroxy-3-methoxyphenyl) octanoic acid (4), 4-(4-hydroxy-3-methoxyphenyl)butanoic acid (5), 9-hydroxy [6]-gingerol (6) were isolated from the bile and urine of gingerol-administered rats. The identity of the compounds were confirmed by [α]D, MS, 1H-, and 13C-NMR spectroscopies before use [17]. Racemic gingerdiol was synthesized by reduction of [6]-gingerol in absolute ethanol with sodium borohydride. Vanillic and ferulic acids, and phthalylsulphathiazol were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). β-Glucuronidase Type H-2, glucose-6-phosphate, uridine 5’-diphosphoglucuronic acid (UDPGA) and neomycin sulfate were purchased from Sigma (St. Louis, MO, U.S.A.). Glucose-6-phosphate dehydrogenase was from Oriental Yeast Co., Ltd. (Tokyo, Japan). For column chromatography, Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden) and Wakogel C-200 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used. All other reagents were of the highest purity commercially available.

Apparatus

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured with a Perkin Elmer FT-IR1725X spectrometer. Optical rotations were in methanol using a Jasco DIP-360 digital polarimeter (cell length: 10 mm). CD spectra were obtained with a JASCO ORD/CD J-20 unit (cell length: 0.2 mm). UV spectra were taken on a Beckman DU-54 spectrometer. NMR spectra were recorded on a JEOL JNM-EX 400 (1H: 400, 13C: 100 MHz) spectrometer. Chemical shifts were given in
δ value (ppm) downfield relative to tetramethylsilane. Electron impact (EI) and FAB-MS were measured with a JEOL JMS-DX 303 mass spectrometer. The HPLC system was comprised of a CCPM pump, CO-8010 column oven (Tosoh, Tokyo) and model MCPD-3600 photodiode array detector (Otsuka, Osaka).

**HPLC conditions**

For qualitative and quantitative analysis of metabolites, HPLC conditions were as follows: column, TSK gel ODS-120T (Tosoh, Tokyo, 250×4.6 mm i.d.); column temperature, 40 °C; flow rate, 1 ml/min; detection, by UV at 200–400 nm. The mobile phase was a gradient system with 0.1% trifluoroacetic acid in H2O (A) and CH3CN (B). The gradient system of qualitative and quantitative analysis were A/B = 100/0 (0 min) → 40/60 (60 min) and A/B = 90/10 (0 min) → 85/15 (40 min) → 30/70 (70 min), respectively.

**Animals**

Male Sprague-Dawley rats (200–250 g) were purchased from Japan SLC, Inc. and five groups (n = 6, for analysis of bile sample, n = 6, for analysis of urine sample, n = 6, for gut sterilization, n = 10, for isolation of metabolites, and n = 3, for collection of liver) of rats were randomly selected for following experiments. These animals were housed in a temperature- (22 ± 2 °C), humidity- (55 ± 10%), and light- (8:00–20:00) controlled room with free access to distilled water and commercial rodent chow (CE-2, Clea Japan Inc., Tokyo). After 7 days of feeding, food was withheld for 18 h and thereafter [6]-gingerol (50 mg/kg body weight) uniformly dispersed in 0.5% Tween 80 was orally administered by direct stomach intubation in a constant volume of 2 ml/kg body weight. The animals were allowed free access to water and sugar during the experiments.

**Bile sample**

After the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (20 mg/kg), their bile ducts were cannulated with polyethylene tubing (i.d. 0.28 mm, o.d. 0.61 mm, Natume, Tokyo, Japan). After the abdominal incision was closed with sutures and wound clipping, [6]-gingerol (50 mg/kg) was orally administered. The rats were then housed in standard Bollman restraining cages. A bile sample was collected in methanol with cooling for 60 h at 12-h intervals. The solution (20 μl), filtered through a 0.45 μm filter membrane, was injected into the HPLC.

**Urine sample**

The urine samples were collected for 60 h at 12 h intervals after the oral administration of [6]-gingerol (50 mg/kg) to the rats by using a metabolic cage. Methanol (5 ml) was added to the urine (3–5 ml) and the mixture was filtered through a 0.45 μm membrane filter, and then 20 μl of the sample was injected into the HPLC.
Enzymatic hydrolysis of bile and urine

Bile or urine sample without methanol was transferred to a test tube to which was added 5.0 ml of 0.2 M sodium acetate buffer (pH 5.5) and 50 μl of β-glucuronidase solution followed by incubation at 37 °C for 24 h. The incubated solution was extracted three times with ethyl acetate (40 ml). The organic layer was then evaporated to dryness at 40 °C. The residue was dissolved in 1 ml of methanol. A 20 μl aliquot was injected into the HPLC.

Gut sterilization

Gut sterilization was done according to the method of Goodwin et al. [18]. Briefly, rats were given orally a mixture of neomycin sulfate (45 mg) and phthalysulphathiazol (0.5 mg) for four days. After 60 min of last dosage on 4 st days, [6]-gingerol (50 mg/kg) was administered to the rats. Urine samples were collected for 24 h and prepared as described above. Statistical analysis was carried out by means of Student’s *t*-test (n = 6). P values less than 0.05 were considered to indicate statistical significance.

Calibration curves

Calibration curves were prepared by spiking predose bile and urine with known amounts of 1, 2, 3, 4, 5, 6, and 7 in the final concentration ranges of 3.50 μg/ml–1.05 mg/ml, 0.285 μg/ml–0.285 mg/ml, 0.354 μg/ml–0.354 mg/ml, 0.539 μg–0.539 mg/ml, 0.710 μg/ml–0.355 μg/ml, 1.26 μg/ml–0.194 mg/ml, and 0.365 μg/ml–0.365 m/ml, respectively. The concentrations of 1–7 were determined from the peak area using the equation for linear regression obtained from the calibration curves.

Recovery

To blank bile or urine were added known amounts of the metabolites (1–7), then these metabolites in the bile or the ethyl acetate extract of the urine were determined. The recoveries of the metabolites were calculated by comparing the experimental values with the corresponding theoretical values.

Isolation of biliary and urinary metabolites

For the biliary and urinary metabolites isolation, rat bile (about 100 ml) and rat urine (about 400 ml) were collected as described above after the [6]-gingerol administration. The bile was successively subjected to Sephadex LH-20 with H2O as the eluent, and the fraction containing 1 was evaporated to dryness under reduced pressure at 40 °C. The residue was further purified by prep. HPLC under the following condition: column, TSKgel ODS-120T (300 × 7.8 mm i.d., Tosoh Company Ltd., Tokyo, Japan); mobile phase, H2O (solvent A) and methanol (solvent B), linear gradient system, A/B = 100/0 (0 min) → 50/50 (50 min). Flow rate was 2.0 ml/min at room temperature. The metabolite fraction was evaporated to dryness
at 40 °C in vacuo to give 1 (30 mg). The urine sample incubated with β-glucuronidase was extracted with ethyl acetate (800 ml) three times. The organic layer was evaporated to dryness at 40 °C. The residue was dissolved in small amount of methanol and chromatographed on Sephadex LH-20 with 50% methanol as the eluant. The fractions containing metabolites (2–7) were subjected to prep. HPLC described above condition. Each metabolite fraction was evaporated to dryness at 40 °C in vacuo to afford 2 (2 mg), 3 (2 mg), 4 (3 mg), 5 (15 mg), 6 (6 mg), and 7 (4 mg), respectively. Metabolite 1: Yellow powder, mp 157–158 °C, [α]D20

−19.04° (c = 0.1, CH3OH). CD (c = 7.90×10−3, CH3OH) [0] (nm) +7800 (248), 0 (254), −95250 (276). UV λ max (CH3OH) nm (log ε): 203 (4.40), 276 (3.38). IR (KBr) cm−1: 3405, 1707, 1514. 1H-NMR (DMSO-d6, 400 MHz) δ: 0.86 (3H, t, J = 7.1 Hz, H-10), 1.24–1.32 (8H, m, H-6–H-9), 2.43–2.45 (2H, m, H-4), 2.70 (2H, m, H-2), 2.76 (2H, m, H-1), 3.08–3.30 (4H, m, H-2–5), 3.75 (3H, s, -OCH3), 3.86 (1H, m, H-5), 4.77 (1H, d, J = 7.6 Hz, H-1), 6.67 (1H, dd, J = 8.3, 2.0 Hz, H-6), 6.82 (1H, d, J = 2.0 Hz, H-2'), 6.96 (1H, d, J = 8.3 Hz, H-5'). 13C-NMR (DMSO-d6, 100 MHz) δ: 13.5 (C-10), 21.6 (C-9), 24.2 (C-7), 28.0 (C-1), 30.8 (C-8), 36.7 (C-6), 43.9 (C-2), 49.9 (C-4), 55.1 (–OCH3), 66.1 (C-5), 71.5 (C-4), 72.5 (C-2), 72.9 (C-5), 76.4 (C-3), 99.9 (C-1), 112.2 (C-2'), 115.3 (C-5'), 119.4 (C-6'), 134.4 (C-1'), 144.2 (C-3'), 148.2 (C-3'), 171.0 (C-6), 208.3 (C-3). HR-FAB-MS m/z: 469.2470 (calcd for C23H32O10: 469.2152 [M–H]−). FAB-MS m/z: 469[M–H]−, 293[M–H–GlcUA]−.


+18.87° (c = 0.1, CH3OH). CD (c = 6.42×10−3, CH3OH) [0] (nm) +26175 (213), 0 (252), −24995 (289). UV λ max (CH3OH) nm (log ε): 206 (3.97), 226 (3.70)(sh), 281 (3.35). IR (neat) cm−1: 3363, 1697, 1667, 1631, 1518. 1H-NMR (CD3OD, 400 MHz) δ: 1.68–1.75 (2H, m, H-3), 2.27 (2H, dd, J = 6.3, 6.3 Hz, H-2), 2.53–2.55 (2H, m, H-5), 2.76–2.79 (4H, m, H-7, 8), 3.82 (3H, s, –OCH3), 4.03–4.08 (1H, m, H-4), 6.61 (1H, dd, J = 8.1, 2.0 Hz, H-6'), 6.68 (1H, d, J = 8.1 Hz, H-5'), 6.77 (1H, d, J = 2.0 Hz, H-2'). 13C-NMR (CD3OD, 100 MHz) δ: 30.3 (C-8), 34.9 (C-3), 35.5 (C-2), 46.4 (C-7), 49.7 (C-5), 56.4 (–OCH3), 69.3 (C-4), 113.2 (C-2'), 116.2 (C-5'), 121.7 (C-6'), 134.2 (C-1'), 145.7 (C-4'), 148.9 (C-3'), 180.5 (C-1), 211.9 (C-6). HR-EI-MS m/z: 296.1211 (calcd for C15H20O6: 296.1260). Metabolite 5: White powder, mp 84 °C. UV λ max (CH3OH) nm (log ε): 202 (3.39), 280 (2.41). IR (KBr) cm−1: 3519, 1695, 1519. 1H-NMR (CDCl3, 400 MHz) δ: 1.93 (2H, m, H-3), 2.37 (2H, t, J = 7.6 Hz, H-2), 2.60 (2H, t, J = 7.6 Hz, H-4), 3.87 (3H, s, –OCH3), 6.66 (1H, d, J = 8.5 Hz, H-6'), 6.67 (1H, s, H-2'), 6.83 (1H, d, J = 8.5, H-5'). 13C-NMR (CDCl3, 100 MHz) δ: 26.5 (C-3), 33.3 (C-2), 34.8 (C-4), 55.95 (–OCH3), 111.0 (C-2'), 114.3 (C-5'), 121.1 (C-6'), 133.1 (C-1'), 143.9 (C-4'), 146.4 (C-3'), 179.4 (C-1). HR-EI-MS m/z: 210.0839 (calcd for C11H14O4: 210.0892).

Metabolite 6: Yellow oil, [α]D20

+14.95° (c = 0.6, CH3OH). CD (c = 1.94×10−2, CH3OH) [0] (nm) +13096 (232), 7196 (284). UV λ max (CH3OH) nm (log ε): 208 (4.08), 226 (3.89)(sh), 281 (3.49). IR (neat) cm−1: 3423, 1704, 1604, 1516. 1H-NMR (CDCl3, 400 MHz) δ: 1.19 (3H, d, J = 6.1 Hz, H-10), 1.33–1.65 (6H, m, H-6–8), 2.49 (1H, dd, J = 8.3, 17.3 Hz, H-4), 2.56 (1H, dd, J = 3.4, 17.3 Hz, H-4), 2.73 (2H, t, J = 6.8 Hz, H-2), 2.83 (2H, t, J = 6.8 Hz, H-1), 3.78–3.83 (1H, m, H-9), 3.87 (3H, s, –OCH3), 4.02–4.04 (1H, m, H-5), 6.65 (1H, dd, J = 8.1, 2.0 Hz, H-6'), 6.68 (1H, d, J = 2.0 Hz, H-2'), 6.82 (1H, d, J = 8.1, H-5'). 13C-NMR
(CDCl₃, 100 MHz) δ: 23.6 (C-10), 24.5 (C-6 or 7), 29.3 (C-1), 29.9 (C-6 or 7), 38.8 (C-8), 45.4 (C-2), 49.4 (C-4), 55.9 (–OCH₃), 67.6 (C-5), 67.9 (C-9), 111.1 (C-2'), 114.5 (C-5'), 120.8 (C-6'), 132.6 (C-1'), 144.0 (C-4'), 146.6 (C-3'), 211.5 (C-3). HR-EI-MS m/z 310.1768 (calcd for C₁₇H₂₆O₅: 310.1780). Metabolite 7: Yellow oil: [α]D₂₀ +29.7° (c = 0.1, CH₃OH). CD (c = 1.81×10⁻², CH₃OH) [θ] (nm) +16075 (234), 0 (255), −17025 (291). HR-EI-MS m/z: 294.1804 (calcd for, C₁₇H₂₆O₄: 294.1831).

Incubation of [6]-gingerol with rat liver

Three rats were sacrificed by decapitation and the livers were immediately removed and perfused in situ with 0.154 M KCl solution. Rat hepatic 10,000×g supernatant was prepared according to the method of Boutin et al. [19]. The incubation flask contained 80 ml of the supernatant, 0.1 mM [6]-gingerol dissolved in 0.2 ml of methanol, the NADPH-generating-system (1.3 mM NADP⁺, 40 unit of glucose-6-phosphate dehydrogenase, 0.3 mM glucose-6-phosphate), 0.3 mM MgCl₂, and 0.1 M PBS (pH 7.4) to a final volume of 200 ml. The incubation was carried out for 1 h at 37°C. The mixture was extracted three times with ethyl acetate (100 ml). The organic layer was then evaporated to dryness at 40°C. The residue was dissolved in 1 ml of methanol and the mixture was filtered through a 0.45 µm membrane filter, and then 20 µl of the sample was injected into the HPLC. For the isolation of the metabolites, the methanol extract was purified by silica gel column chromatography (n-hexane-ethyl acetate 5:1) to give 6 (0.3 mg) and 8 (3 mg). Metabolite 8: Yellowish viscous solid, mp. 67–68°C. [α]D₂₀ −0.47° (c = 0.2, CH₃OH). HR-EI-MS m/z: 296.1991 (calcd for C₁₇H₂₈O₄: 296.1988). The incubation flask contained 5 ml of rat hepatic 10,000×g supernatant, 10 mM MgCl₂, 0.25 mM Triton X-100, 52 mM UDPGA, and 0.1 mM [6]-gingerol dissolved in 20 µl of ethanol. The incubation was carried out for 1 h at 37°C. The reactions were terminated by adding 15 ml ice-cold methanol, followed by centrifugation at 4°C. The supernatants were then evaporated to dryness. The residue was dissolved in 1 ml methanol, the mixture was filtered through a 0.45 µm membrane filter, and then 20 µl of the sample was injected into the HPLC.

Results

Identification of metabolites

By using an HPLC equipped with a photodiode array detector, a main metabolite (1) was detected in the bile sample after the oral administration of [6]-gingerol (50 mg/kg) as shown in Fig. 1. The treatment of the bile sample with β-glucuronidase led to the disappearance of 1 and the concomitant appearance of [6]-gingerol. On the other hand, metabolites derived from [6]-gingerol were not detected in the urine sample (data not shown), but the analysis of the ethyl acetate extract of the urine sample obtained after incubation with β-glucuronidase revealed at least 6 minor metabolites (2–7). The typical chromatogram of the ethyl acetate extract of the urine sample is shown in Fig. 2. The retention times for the metabolites were as
follows: 1 – 39 min, 2 – 19 min, 3 – 24 min, 4 – 25 min, 5 – 29 min, 6 – 31 min, and 7 – 49 min. The structures of these metabolites were identified as (S)-[6]-gingerol-4′-O-β-glucuronide (1), vanillic acid (2), ferulic acid (3), (S)-(+)−4-hydroxy-6-oxo-8-(4-hydroxy-3-methoxyphenyl)octanoic acid (4), 4-(4-hydroxy-3-methoxyphenyl)butanoic acid (5), 9-hydroxy...
[6]-gingerol (6) and (S)-(−)-[6]-gingerol (7), respectively, by direct comparison of the UV spectra and retention time with those of authentic samples. The HPLC chromatograms of the ethyl acetate (NADPH-generating-system) and methanol extract (UDPGA) obtained after the incubation of [6]-gingerol with rat liver showed the presence of two metabolites and a metabolite, respectively (chromatogram not shown). These metabolites were identified as 9-hydroxy [6]-gingerol (6) and gingerdiol (8) and (S)-[6]-gingerol-4′-O-β-glucuronide (1), respectively, by comparisons of the spectral data with those of authentic samples. Based on the integrated peak areas, 6, 8, and 1 represented approximately 1%, 43%, and 10% of the added [6]-gingerol formed during the incubations, respectively.
Quantification of metabolites

The peak areas were linearly related to the concentrations of 1–7 and the equations for the regression lines for 1–7 were found to be 1: \( y = 13.731x + 0.007 \) (\( r = 0.999 \)), 2: \( y = 1.559x + 0.001 \) (\( r = 1.000 \)), 3: \( y = 0.535x \) (\( r = 1.000 \)), 4: \( y = 7.893x + 0.003 \) (\( r = 1.000 \)), 5: \( y = 5.287x \) (\( r = 1.000 \)), 6: \( y = 8.839x + 0.004 \) (\( r = 0.999 \)), 7: \( y = 5.327x + 0.008 \) (\( r = 1.000 \)). The detection limits (signal to noise ratio = 4) of 1–7 were 0.857 mg/ml, 0.081 mg/ml, 0.035 mg/ml, 0.238 mg/ml, 0.273 mg/ml, 0.268 mg/ml, and 0.258 mg/ml in rat bile and urine, respectively. The recoveries of 1–7 from rat bile and urine were found to be 90.36–98.55%. The biliary excretion profile of metabolite 1 after administering 50 mg/kg of [6]-gingerol is shown in Fig. 3. The total cumulative amounts of 1 excreted during 60 h corresponded to 48.44 ± 2.47% (mean ± S.E., n = 5) of the dose for [6]-gingerol administration. The urinary excretion of metabolites (2–7) is shown in Fig. 4. The cumulative amounts of 2–7 excreted into the urine after 60 h corresponded to 1.15 ± 0.24, 0.11 ± 0.01, 4.30 ± 0.65, 5.11 ± 0.77, 3.03 ± 0.33, and 2.31 ± 0.23% of [6]-gingerol administration, respectively, and the total cumulative mean was 16.01 ± 2.24%. The effect of gut flora sterilization on the urinary metabolites is shown in Table 1. The urinary excretion of 4–7 during 24 h after [6]-gingerol administration significantly decreased after treatment with neomycin (20 mg) and phthalylsulphathiazol (0.5 mg). The excretions of 2 and 3 were lower when compared with the untreated rats, but the values were not significant.

Discussion

The rhizome of ginger is one of the most important crude drugs in traditional Chinese medicine, and contains [6]-gingerol as an effective constituent. Generally, traditional medicines are orally administered, and the components in the crude drugs may be metabolized by gut flora before being absorbed into the body. Accordingly, to evaluate the bioactive compounds in these crude drugs, an investigation of the compounds actually absorbed into body is necessary, and knowledge of the metabolism of the compounds may help in understanding the mechanism of action and therapeutic effects of these crude drugs. Although, there have been a few reports on the metabolism of [6]-gingerol, they do not
provide information the metabolites of orally administered [6]-gingerol in mammals. In this study, we isolated (S)-[6]-gingerol-4′-O-β-glucuronide (1) from the bile after the oral administration of [6]-gingerol to rats and vanillic acid (2), ferulic acid (3), (S)-(+)4-hydroxy-6-oxo-8-(4-hydroxy-3-methoxyphenyl)octanoic acid (4), 4-(4-hydroxy-3-methoxy-phenyl)butanoic acid (5), 9-hydroxy [6]-gingerol (6), and (S)-(+)-[6]-gingerol (7) from the β-glucuronidase-treated urine. Thus, [6]-gingerol orally administered to rats undergoes conjugation, and ω-1 oxidation and β-oxidation of a phenolic side chain. Quantification of these metabolites indicated that the excretions of 1–6 continued up to 36–48 h, and approximately 48% of the dose was excreted in the bile as glucuronide (1). The total cumulative amounts of 2–7 into the urine were approximately 16% of the dose. In general, orally administered phenolic compounds, especially those with a low polarity, undergo hydroxylation and/or glucuronide and sulfate conjugation primary in the intestinal mucosa and secondarily in the liver and other tissues. We also previously reported that paeonol (2-hydroxy-4-methoxyacetophenone) orally administered to rats was excreted into the bile and urine as four sulfated metabolites [20]. However, in the present study, we could not any detect sulfates in the bile and urine after [6]-gingerol administration. From these results, we speculate that [6]-gingerol is a high affinity substrate for glucuronidation rather than sulfation, and that its properties, the high molecular weight and high polarity of glucuronide, promote the excretion of [6]-gingerol in the bile (1 in bile/7 in urine 21:1). The contribution of the liver to the metabolism of [6]-gingerol was examined using the rat hepatic 10,000 g supernatant in the presence of the NADPH-generating system or UDPGA. The HPLC chromatograms of the reaction mixtures were different from those of the in vivo experiments, namely, when the NADPH-generating system or UDPGA were present in the reaction mixture, 6 and gingerdiol or 1 were formed, respectively. However, 2–5 were not detected and the formation of 1 was only about 10% in the reaction mixtures. Surh et al. have also reported that gingerdiol was formed by the enzymic reduction of [6]-gingerol in a cell-free preparation of the rat liver [16]. These results suggest that the liver is one of the organs, which take part in the formation of 1 and 6 from [6]-gingerol (glucuronidation and ω-1 oxidation), although other majority biotransformations were conducted elsewhere, for example, intestinal microflora and mucosa. In order to determine the contribution made by intestinal microflora to the formation of 2–6, we next examined the effect of gut sterilization on the urinary metabolite excretion. The excretion of 2–6 in urine decreased after gut sterilization. This result demonstrated the importance of gut flora in generating urinary metabolites 2–6. Asai et al. also reported that in spite of the UDP-glucuronosyltransferase activity in the liver being higher than that in the intestinal mucosa, the majority of the orally administered curcuminoid, a phenolic compound of turmeric, were conjugated to glucuronide in the intestinal mucosa [21]. Therefore, it may be considered that 1 was formed not only in the liver but also in the intestinal mucosa. According to an early study by Suekawa et al., the pharmacological effect was maintained until 120 or 180 min after the i.v. and p.o. administrations of [6]-gingerol [8]. However, Ding et al. showed that the plasma concentration of [6]-gingerol at 30 min was less than about 0.1% of that immediately attained after i.v. administration (3 mg/kg), and the [6]-gingerol was cleared very rapidly from the plasma with a short terminal half-life (7.23 min) in rats [14]. With respect to the discrepancy or time-shift between the pharmacological effect and plasma
level due to the rapid clearance, they deduced that some active metabolites may be produced or [6]-gingerol may be effective at a much lower plasma concentration than those detected by the assay [14]. Although, the pharmacological activity of 1–6 has not yet been studied, these metabolites may help in understanding the mechanism of these actions and the therapeutic effects of ginger because these metabolites at least stay in body over 12 h after administration. Further detailed work is necessary to clarify the metabolite formation and the pharmacological activity of these metabolites. These studies are now in progress.

References