Research Article

Examination of the Pharmacokinetics of Active Ingredients of Ginger in Humans

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Abstract. Ginger extracts have been studied in various clinical trials for different indications. However, the pharmacokinetics of the ginger active constituents in human biological matrices is not well investigated. This study aims to develop a LC-MS/MS method for simultaneous measurement of 6-, 8-, and 10-gingerols and 6-shogaol and study their pharmacokinetics in human plasma and colon tissues. A sensitive LC-MS/MS method was established and validated with a low limit of quantification of 2–5 ng/mL. The intra- and inter-day accuracy ranged from –7.3% to 10.4% and from –9.4% to 9.8%, respectively. The intra- and inter-day precision ranged from 0.9% to 10.9% and from 2.0% to 12.4%, respectively. The glucuronide and sulfate metabolites of 6-, 8-, and 10-gingerols and 6-shogaol in plasma and colon tissues were quantified after hydrolysis with β -glucuronidase and sulfatase. After oral dosing of 2.0 g ginger extracts in human, free 10-gingerol and 6-shogaol were detected in plasma with peak concentrations (9.5 ± 2.2 and 13.6 ± 6.9 ng/mL, respectively) at 1 h after oral administration, but no free 6-gingerol and 8-gingerol were detected in plasma from 0.25 to 24 h. The peak concentrations of glucuronide metabolites of 6-, 8-, and 10-gingerols and 6-shogaol were 0.47 ± 0.31 , 0.17 ± 0.14 , 0.37 ± 0.19 , and 0.73 ± 0.54 μ g/mL at 1 h, respectively. The peak concentrations of the sulfate metabolites of 6-, 8-, and 10-gingerols and 6-shogaol were 0.28 ± 0.15 , 0.027 ± 0.018 , 0.018 ± 0.006 , and 0.047 ± 0.035 μ g/mL at 1 h, respectively. Very low concentrations (2–3 ng/mL) of 10-gingerol glucuronide and sulfate were found in colon tissues. Pharmacokinetic analysis showed that half-lives of these four analytes and their metabolites were 1–3 h in human plasma. No accumulation was observed for 6-, 8-, and 10-gingerols and 6-shogaol and their metabolites in both plasma and colon tissues after multiple daily dosing.

KEY WORDS: 6-Gingerol; 8-Gingerol; 10-Gingerol and 6-shogaol; LC-MS/MS; glucuronide; sulfate; pharmacokinetics.

INTRODUCTION

The antioxidative, anti-inflammatory, and antitumor properties of ginger (*Zingiber officinale* Roscoe, Zingiberaceae) have been reported in previous studies (1–5). Various clinical trials have evaluated ginger extracts to decrease lipid levels (6), treat arthritis (7,8), prevent nausea and vomiting (9–11), and reduce pain in women with primary dysmenorrhea (12). However, clinical studies using ginger extracts produced mixed or moderate/marginal benefits. For instance, Chaiyakunapruk *et al.* (13) demonstrated that administration of ginger at a dose of 1 g was more effective than placebo for the prevention of postoperative nausea and vomiting and postoperative vomiting. In contrast, Betz *et al.* (14) concluded that there was no clear evidence for the efficacy of ginger in the treatment of postoperative nausea and vomiting and of kinetosis. Bliddal *et al.*

(15) showed that 170-mg daily dosing for 3 weeks of ginger powder did not show significant benefit over placebo in relieving pain in patients with osteoarthritis. However, a 6-week treatment with 510 mg of ginger extract daily dosing produced moderate effect on reducing pain in the knee in patients with osteoarthritis (16).

Ginger contains volatile oils (~1% to 3%) and non-volatile pungent components oleoresin (1). A variety of active components were identified in the oleoresin of ginger including gingerols and shogaols. Gingerols are a series of homologues with varied unbranched alkyl chain length, whereas shogaols are a series of homologues derived from gingerols with dehydration at the C-5 and C-4 during long-term storage or thermal processing. Other active compounds from the oleoresin portion of ginger were also reported, such as [6]-paradol; [6]- and [10]-dehydrogingerdione; [6]- and [10]-gingerdione; [4]-, [6]-, [8]-, and [10]-gingerdiol; [6]-methylgingerdiol; zingerone; [6]-hydroxshogaol; [6]-, [8]-, and [10]-dehydroshogaol; and diarylheptanoids (17–19). Among these compounds, gingerols and shogaols are the major constituents of oleoresin, while the other compounds are present in a limited amount, accounting for 1–10% of the overall amount of gingerols and shogaols (19). Gingerols (especially 6-gingerol) are the major components in the fresh ginger rhizome. The amount of shogaols is increased in the

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dried ginger, as evidenced by the reduction of the ratio of 6-gingerol to 6-shogaol from 10:1 in fresh ginger to 1:1 in dried ginger (17,18,20).

Since ginger extracts contain various components, it would be important to identify which compounds are responsible for their pharmacological effects. It was demonstrated that 6-, 8-, and 10-gingerols and 6-shogaol showed efficacy in anti-inflammatory, antibacterial, antipyretic, antilipidemic, antitumorigenic, and antiangiogenic effects (5,21–27). In addition, 6-gingerol was shown to inhibit leukotriene A₄ hydrolase (LTA₄H) and suppress anchorage-independent cancer cell growth in colorectal cancer cells (HCT116 and HT29) with IC₅₀'s of 50 and 35 μ M, respectively (28). Sang *et al.* (19) demonstrated that 6-, 8-, and 10-shogaols exhibited much higher antiproliferative potency than 6-, 8-, and 10-gingerols against human lung cancer cells (H-1299) with IC₅₀'s of 8 μ M for 6-shogaol and 150 μ M for 6-gingerol. In addition, 10-gingerol was the most potent among the gingerols (19). Furthermore, Dugasani *et al.* (29) found that 6-shogaol showed the most potent efficacy of antioxidative activity with an IC₅₀ of about 8 μ M, while 6, 8, and 10-gingerols had IC₅₀'s of 28, 20, and 12 μ M, respectively.

In most of the clinical trials, the content of active ingredients in the ginger extract was not measured. The use of non-standardized ginger extracts in different clinical studies partly explain the mixed results of the clinical studies in addition to the different study designs and dose regimens. A study by Schwertner *et al.* showed that variable amount of 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol in various brands of ginger root dietary supplements (7). In these different products, 6-gingerol ranged from 0.00 to 9.43 mg/g, 6-shogaol ranged from 0.16 to 2.18 mg/g, 8-gingerol ranged from 0.00 to 1.10 mg/g, and 10-gingerol ranged from 0.00 to 1.40 mg/g.

Furthermore, despite the numerous studies of the pharmacological effects of the ginger extracts in the human clinical trials, there are limited studies of the pharmacokinetics of the ginger active constituents in human biological matrices. The concentrations of 6-, 8-, and 10-gingerols and 6-shogaol for their efficacy *in vivo* are still largely unknown. In our previous study, we developed a HPLC method to determine the concentrations of 6-, 8-, and 10-gingerols and 6-shogaol in healthy human subjects who received oral dose of ginger extracts at 2.0 g per day (1). However, due to the low sensitivity of the HPLC method with low limit of quantification (LLOQ) ranging from 0.10 to 0.25 μ g/mL for the four analytes, we did not detect any of these four compounds in the plasma, although we detected the glucuronide conjugates of the four analytes. No sulfate conjugates of 8-gingerol, 10-gingerol, and 6-shogaol were detected. Thus, a more sensitive method for the quantification of 6-, 8-, and 10-gingerols and 6-shogaol and their metabolites is desired to characterize the pharmacokinetics of the active ingredients of ginger in human.

In this study, we developed and validated a LC-MS/MS method for the quantification of 6-, 8-, and 10-gingerols and 6-shogaol simultaneously, with the LLOQ ranging from 2 to 5 ng/mL. We further utilized this method to analyze human plasma samples and detected low concentrations of free 10-gingerol and 6-shogaol, while most of the 6-, 8-, and 10-

gingerols and 6-shogaol existed in plasma as glucuronide and sulfate metabolites. The pharmacokinetics of 6-, 8-, and 10-gingerols and 6-shogaol and their metabolites were analyzed. The half-lives of all compounds and their metabolites were between 1 and 3 h (Fig. 1).

MATERIALS AND METHODS

Chemicals and Reagents

The ginger product used in this study was manufactured by Pure Encapsulations® (Sudbury, MA; batch no. ZO/06006). A 250-mg dry extract of ginger root, which contained 6.60 mg (2.64%) 6-gingerol, 1.58 mg (0.63%) 8-gingerol, 3.05 mg (1.22%) 10-gingerol, and 5.63 mg (2.25%) 6-shogaol, was used. β -glucuronidase (type IX-A from *Escherichia coli*) and sulfatase (type H-1 from *Helix pomatia*) were purchased from Sigma-Aldrich Inc. Sodium phosphate and sodium acetate (American Chemical Society-certified) were purchased from Fisher Scientific. The 6-, 8-, and 10-gingerols and 6-shogaol were purchased from Chromadex. Pelargonic acid vanillylamide (PAV), the internal standard, was obtained from Sigma. Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Fisher Scientific Co. (Pittsburgh, PA). Formic acid (analytical grade) was from Sigma Chemical Company (St. Louis, MO). Water was purified with a Milli-Q water system (Millipore, Bedford, MA).

Clinical Trial Design

Three studies were conducted in healthy volunteers, single dose in normal-risk participants, and multiple doses in normal-risk and high-risk participants. Participants were 18 years or older and in good health as defined by an unremarkable medical history, physical and screening blood work, and no chronic medication use. Exclusion criteria for the study included: anyone with (1) a history of peptic ulcer disease, gastrointestinal bleeding from gastric or duodenal ulcers, or gastrin secreting tumors; (2) pregnant or lactating women; (3) history of cardiovascular disease; (4) lactose intolerance; (5) or an allergy to ginger. Participants were asked to avoid all foods containing ginger within the 14 days prior to drug administration and completed a food checklist to verify that they were not consuming any ginger-rich foods

Fig. 1. Chemical structures of 6-, 8-, and 10-gingerols, 6-shogaol, and internal standard pelargonic acid vanillylamide (PAV)

such as ginger ale or Japanese food. All study procedures were performed at the University of Michigan General Clinical Research Center after the participant gave written and informed consent. The study was approved by the University of Michigan Institutional Review Board.

Single Dose in Healthy Volunteers

Nine healthy volunteers received 2-g oral dose of the ginger extracts. Blood was drawn from the participants at baseline, 0.25, 0.5, 0.75, 1, 2, 4, 6, 10, 24, 48, and 72 h after ingestion of the ginger extracts. The plasma was separated from blood immediately and kept at -80°C until LC-MS analysis.

Multiple Doses in Normal-Risk Participants

Thirty participants were enrolled and recruited. Participants to be eligible were assessed as being at normal risk for developing colorectal cancer. Normal risk was defined as no history of either familial colorectal cancer syndromes or first-degree relatives with colon cancer diagnosed before the age of 60. Normal-risk individuals also could not have a personal history of colorectal cancer or resection of a villous adenoma (>1 cm in size or any adenoma containing carcinoma *in situ*). Participants were randomized into two different groups. Sixteen patients were given placebo, while 14 patients were given eight 250-mg ginger extract capsules daily for 28 days. Blood was drawn at baseline and within 24 h after the last dose. The plasma was separated from blood immediately and kept at -20°C until assayed.

Participants underwent two flexible sigmoidoscopies, one before drug treatment and the second at 28 days after drug treatment. The second procedure was performed at 24 h after the final ginger dose. The participants were not prepared for the procedure with any enemas.

Each biopsy specimen was taken ~ 2 cm or more from other biopsy sites in distal sigmoid colonic mucosa that had no visual appearance of trauma or recent biopsy. Biopsy samples were placed into a sterile 1.5-mL Eppendorf tube and frozen in liquid nitrogen. The specimens were stored at -80°C until analysis.

Multiple Doses in High-Risk Subjects

Twenty participants who were at high risk for developing colorectal cancer were registered in the study. Participants were randomized, ten in each group, to receive either eight 250-mg ginger extract capsules or placebo (lactose powder) daily for 28 days. High risk for colorectal cancer was defined as having a first-degree relative diagnosed with colon rectal cancer before the age of 60, and/or a prior history of a colon adenoma, and/or resected early (Dukes A, B, or C) colon cancer previously.

Equipment

The quantitative LC-MS/MS analysis was performed on an API 3200 hybrid triple quadrupole/linear ion trap mass

spectrometer coupled with an Agilent 1200 HPLC system (Applied Biosystems, MDS Sciex Toronto, Canada).

Liquid Chromatography Parameters

The chromatography was performed using a $1.8\text{-}\mu\text{m}$ Agilent Zorbax StableBond- C_{18} column ($4.6 \times 50\text{-mm}$ i.d.). The injection volume was $10\text{ }\mu\text{L}$ and the flow rate was $300\text{ }\mu\text{L}/\text{min}$. Mobile phase A and B were water containing 0.1% formic acid (v/v) and ACN, respectively. The flow gradient was initially at 38:62 (v/v) of A/B for 3 min, linearly ramped to 0:100 over 1.5 min, held at 0:100 for 3.4 min, and then returned to 38:62 for 0.1 min. This condition was held for a further 5 min prior to the injection of another sample.

MS/MS Parameters

The mass spectrometer was operated at ESI positive ion mode and detection of the ions was performed in the multiple reaction monitoring (MRM) mode. The analytes and internal standard (IS) were first characterized by Q1 MS (Q1) scan and enhanced product ion (EPI) scan to determine the precursor ions and product ions used in MRM mode. Figure 2 shows the EPI spectra of the analytes and IS. The MS/MS transitions were: 6-gingerol, m/z 295.2 precursor ion $[\text{M}^+\text{H}]^+$ to the m/z 137.1 product ion; 8-gingerol, m/z 323.2 precursor ion $[\text{M}^+\text{H}]^+$ to the m/z 137.1 product ion; 10-gingerol, m/z 351.2 precursor ion $[\text{M}^+\text{H}]^+$ to the m/z 137.1 product ion; 6-shogaol, m/z 277.2 precursor ion $[\text{M}^+\text{H}]^+$ to the m/z 137.1 product ion; IS, m/z 294.2 precursor ion $[\text{M}^+\text{H}]^+$ to the m/z 137.1 product ion. The ion spray voltage was set at 5,000 V, ionization temperature set as 400°C , CAD was set as medium, and the was set as OFF. The instrument parameters and curtain gas, gas 1 and gas 2 (auxiliary gas), were set at 10, 60, and 40, respectively. Compound parameters, declustering potential, collision energy, entrance potential, collision entrance energy, and collision exit potential were 33, 27, 5, 18.4, and 2 V for 6-gingerol; 33, 27, 5, 19.19, and 2 V for 8-gingerol; 33, 27, 5, 19.97, and 2 V for 10-gingerol; 44, 18, 5, 17.9, and 2 V for 6-shogaol; and 33, 27, 5, 18.38, and 2 V for internal standard PAV, respectively. Data acquisition and quantitation were performed using analyst software version 1.4.2 (Applied Biosystems, MDS Sciex Toronto).

Preparation of Stock Solution, Calibration Standard, and Quality Control Samples

The stock solutions of 6-, 8-, and 10-gingerols, 6-shogaol, and PAV were prepared in methanol with concentrations of $1\text{ mg}/\text{mL}$. A series of working standard of analyte mixture containing 0.04, 0.1, 0.2, 0.4, 1, 2, 4, 10, and $40\text{ }\mu\text{g}/\text{mL}$ of the four analytes was prepared by dilution from the stock solutions with methanol. Internal standard PAV working solution was prepared by diluting the stock solution with methanol to the final concentration of $2\text{ }\mu\text{g}/\text{mL}$. Low, medium, and high concentration quality control working stock solutions (0.3, 5, and $20\text{ }\mu\text{g}/\text{mL}$) were prepared in

Fig. 2. EPI mass spectra of the analytes and internal standard. **a** EPI of 6-gingerol; **b** EPI of 8-gingerol; **c** EPI of 10-gingerol; **d** EPI of 6-shogaol; **e** EPI of IS

methanol using separately weighed stock solutions of the four analytes. Nine calibration standard solutions at 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 2 $\mu\text{g/mL}$ were prepared by spiking blank plasma with appropriate amounts of working standards. QC plasma samples at 0.015, 0.25, and 1 $\mu\text{g/mL}$ were prepared in the same way as the calibration standard. Blank plasma sample without analytes and internal standard and zero plasma samples with internal standard but no analytes were also prepared and analyzed. All the standard solutions were stored at 4°C.

Sample Preparation

Plasma samples (100 μL) were transferred to micro-centrifuge tubes. Then, 5 μL internal standard PAV working

solution (2 $\mu\text{g/mL}$) was added to the plasma; the mixture was added with 295 μL ACN and vortexed for 1 min at high speed. The tubes were centrifuged at 13,000 rpm for 10 min to precipitate protein. The supernatants were transferred to vial inserts and 10 μL was injected into LC-MS/MS.

To analyze the metabolic conjugates (glucuronide and sulfate) of 6-, 8-, and 10-gingerols and 6-shogaol, the plasma samples were pre-incubated with β -glucuronidase and sulfatase as described previously (1,30). The total concentrations of the gingerols and shogaol with separate enzyme hydrolysis were comparable to those with hydrolysis in mixtures, which indicated the specificity of these enzymes (1). Briefly, 100 μL plasma samples was added with either 10 μL β -glucuronidase (500 U) in sodium phosphate buffer (0.1 M, pH 6.8) or 10 μL sulfatase (60 U) in sodium acetate buffer

Fig. 3. Representative MRM chromatograms of the analytes and internal standard: 6-gingerol, 295.2/137.0; 8-gingerol, 323.2/137.0; 10-gingerol, 351.2/137.0; 6-shogaol, 277.2/137.0; internal standard PAV, 294.2/137.0. **a** Blank plasma sample. **b** blank plasma sample spiked with four analytes and IS. **c** Plasma sample obtained 1 h after oral administration of ginger extracts

(0.1 M, pH 5.0) and incubated at 37°C for 1 h. The samples were then extracted as described above.

Method Validation

Specificity. Specificity of the analytical method was investigated by the analysis of blank plasma samples from six different sources to avoid potential endogenous interferences at the retention times of the analytes and IS.

Extraction Recovery. Extraction recovery was determined in triplicate by comparing the peak areas of analytes determined in QC samples spike-pre-extraction and QC samples spike-after extraction.

Linearity and LLOQ. Linearity was evaluated in triplicate for 6-, 8-, and 10-gingerols in a concentration range from 0.005 to 2 µg/mL while in a concentration range from 0.002 to 2 µg/mL for 6-shogaol. The lower limit of quantification

was determined as the lowest concentrations of the analytes which had a signal-to-noise ratio (S/N) over 10 and had acceptable accuracy within ±20% (% bias) and precision <20% (% RSD).

Accuracy and Precision. Accuracy was calculated as the mean percentage deviation of the measured concentrations of the three QC samples and LLOQ sample from their nominal concentrations. Precision was calculated as the coefficient of variation of multiple determinations. Both the inter-day and intra-day results were determined for the accuracy and precision.

Pharmacokinetic Analysis

Pharmacokinetic analysis of the plasma analytes concentration-time profile was carried out by Winnolin software (Pharsight, Mountain View, CA).

Table I. Calibration Curve, Linear Range, and LLOQ of 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol in Plasma

Compounds	<i>r</i>	Linear range (ng/mL)	LLOQ (ng/mL)
6-Gingerol	0.998	5–2,000	5
8-Gingerol	0.999	5–2,000	5
10-Gingerol	0.999	5–2,000	5
6-Shogaol	0.999	2–2,000	2

RESULTS

LC-MS/MS Analysis

Blank plasma samples from six different sources were analyzed and found to be free of the interference at the retention times and the mass transitions as the analytes and IS. Figure 3 shows the representative chromatograms of the blank plasma, blank plasma spiked with analytes and IS, and real plasma sample obtained 1 h after oral administration of ginger powder capsules. The retention time was 4.03 min for 6-gingerol, 7.16 min for 8-gingerol, 9.42 min for 10-gingerol, 8.35 min for 6-shogaol, and 4.80 min for IS. Despite the isotope of IS resulting in a peak at 4.82 min in the 6-gingerol channel, all the analytes and IS achieved baseline separation from each other.

Linearity and LLOQ

Calibration curves were constructed from the peak area ratios of analytes to IS vs. plasma concentrations using quadratic regression with a 1/*x* weighting. Table I shows the linear range, coefficient (*r*), and LLOQ. The LLOQs were determined as 5 ng/mL for 6-, 8-, and 10-gingerols and 2 ng/mL for 6-shogaol.

Extraction Recovery

Extraction recoveries of the established method ranged from 84.4% to 97.6% for 6-gingerol, from 81.7% to 94.7% for 8-gingerol, from 80.4% to 92.2% for 10-gingerol, and from 81.8% to 93.6% for 6-shogaol, respectively (Table II). Overall, the extraction recovery was efficient and consistent at different concentration levels of analytes.

Accuracy and Precision

The results for intra-day and inter-day accuracy and precision are listed in Table III. The intra- and inter-day

Table II. Extraction Recovery for 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol in Human Plasma, Expressed as Mean ± RSD (%)

QC (ng/mL)	6-Gingerol	8-Gingerol	10-Gingerol	6-Shogaol
15	96.6±7.2	87.1±2.1	82.4±9.0	85.7±0.6
250	84.4±2.4	81.7±4.1	80.4±7.6	81.8±2.9
1000	97.6±2.7	94.7±2.0	92.2±4.8	93.6±3.2

Table III. Precision and Accuracy of LC-MS/MS Analysis of 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol

Analyte	Nominal concentration (ng/mL)	Intra-day				Inter-day			
		Measured concentration (ng/mL)	Precision (% RSD)	Accuracy (% bias)		Measured concentration (ng/mL)	Precision (% RSD)	Accuracy (% bias)	
6-Gingerol	15	16.5	1.0	10.0		16.1	9.0	7.1	
	250	245	2.3	-2.0		249	6.2	-0.1	
	1,000	926	5.7	-7.3		904	3.0	-9.5	
8-Gingerol	15	15.9	2.0	6.2		15.8	4.4	5.3	
	250	235	10.9	-5.9		267	4.0	7.2	
	1,000	941	2.5	-5.8		978	12.4	-3.2	
10-Gingerol	15	16.5	1.3	9.8		15.9	2.0	6.4	
	250	248	2.6	-5.5		274	5.4	9.8	
	1,000	992	5.2	-0.7		1,025	9.4	2.5	
6-Shogaol	15	16.6	0.9	10.4		15.3	4.1	2.2	
	250	242	1.6	-3.3		269	2.4	7.6	
	1,000	966	4.3	-3.4		969	6.5	-3.1	

Fig. 4. Mean plasma concentration–time profiles of 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, and their glucuronide and sulfate conjugates in human plasma after oral administration of 2.0 g ginger powders ($n=8$). **a** 6-Gingerol; **b** 8-gingerol; **c** 10-gingerol; **d** 6-shogaol

accuracy (expressed as percentage bias against the nominal concentration) ranged from -7.3% to 10.4% and from -9.4% to 9.8% , respectively. The intra- and inter-day precision (expressed as RSD) ranged from 0.9% to 10.9% and from 2.0% to 12.4% , respectively. Therefore, the accuracy and precision for this quantification method are acceptable.

Stability

We have tested the stability of the analytes by a re-assay of the samples stored in -80°C for 6 months. Because of the lack of gingerol and shogaol glucuronide or sulfate conjugates standard, we were not able to determine the stability of the conjugates directly. We reanalyzed the concentration of glucuronide and sulfate conjugates of 6-, 8-, and 10-gingerols and 6-shogaol 6 months after our original quantification. The concentrations determined were within 90–110% of the original quantification values determined by using LC-MS/MS. In addition, the concentrations of the 6-, 8-, and 10-gingerols and 6-shogaol conjugate metabolites were within 85–115% of the concentrations determined by HPLC which was performed 1.5 years ago.

Pharmacokinetic Study

Concentrations of 6-, 8-, and 10-Gingerols and 6-Shogaol in the Plasma After the Single Dose of Ginger Powder Capsules

In the present study, we applied the LC-MS/MS method to quantify the concentrations of 6-, 8-, and 10-gingerols and 6-shogaol, as well as their glucuronide and sulfate conjugates. Free 10-gingerol was detected in plasma with a peak concentration of 9.5 ± 2.2 ng/mL at 1 h, which

was undetectable after 2 h post-dosing. Free 6-shogaol was detected in plasma at a peak concentration of 13.6 ± 6.9 ng/mL at 1 h, which was undetectable after 4 h post-dosing (Fig. 4). No free 6-gingerol or 8-gingerol was detected in the plasma samples from 0 to 24 h post-dosing. The terminal half-lives of 10-gingerol and 6-shogaol were 2.1 and 1.3 h, respectively. Other pharmacokinetic parameters were listed in Table IV.

Glucuronide Conjugates of 6-, 8-, and 10-Gingerols and 6-Shogaol in the Plasma After Single Dose of Ginger Powder Capsules

As gingerols were predominantly present in the form of glucuronide conjugates in the plasma samples after oral dosing (1), we further analyzed the glucuronide conjugates of 6-, 8-, and 10-gingerols and 6-shogaol. The plasma samples were first subjected to β -glucuronidase hydrolysis, then followed by liquid–liquid extraction as described previously (1). 6-Gingerol glucuronide conjugate was detected from 0.25 to 10 h with peak concentration of 0.47 ± 0.31 $\mu\text{g/mL}$ at 1 h. 8-Gingerol glucuronide conjugate was observed from 0.25 to 10 h with peak concentration of 0.17 ± 0.14 $\mu\text{g/mL}$ at 1 h. 10-Gingerol glucuronide conjugate was observed from 0.25 to 10 h with peak concentration of 0.37 ± 0.19 $\mu\text{g/mL}$ at 1 h. 6-Shogaol glucuronide conjugate was observed from 0.25 to 8 h with peak concentration of 0.073 ± 0.054 $\mu\text{g/mL}$ at 1 h (Fig. 4). To analyze the pharmacokinetic parameters of glucuronide conjugates, the concentrations were calculated with the subtraction of free analyte concentrations since the concentrations of glucuronides were measured after the conversion of metabolites to free analytes. The pharmacokinetic parameters were summarized in Table IV. The half-lives of

Table IV. Estimated Plasma Pharmacokinetic Parameters After Single Oral Administration of 2.0 g Ginger Extracts

Parameter	6-Gingerol			8-Gingerol			10-Gingerol			6-Shogaol		
	Free	Glucuronide	Sulfate	Free	Glucuronide	Sulfate	Free	Glucuronide	Sulfate	Free	Glucuronide	Sulfate
$AUC_{(0-1)}$ ($\mu\text{g h/mL}$)	N/A	0.74 ± 0.56	0.43 ± 0.26	N/A	0.26 ± 0.27	0.036 ± 0.035	0.008 ± 0.004	0.88 ± 0.60	0.059 ± 0.015	0.024 ± 0.013	0.11 ± 0.08	0.079 ± 0.044
$MRT_{(0-1)}$ (h)	N/A	1.61 ± 0.34	1.77 ± 0.34	N/A	1.34 ± 0.36	1.05 ± 0.36	0.93 ± 0.28	2.04 ± 0.34	2.94 ± 1.03	1.18 ± 0.36	1.48 ± 0.52	1.58 ± 0.40
$T_{1/2}$ (h)	N/A	1.64 ± 0.88	1.79 ± 0.99	N/A	1.03 ± 0.34	1.25 ± 0.26	1.79 ± 0.32	2.09 ± 0.44	3.24 ± 1.83	1.32 ± 0.44	1.54 ± 0.66	1.40 ± 0.36
$T_{\max}$ (h)	N/A	1.03 ± 0.41	1.03 ± 0.41	N/A	1.06 ± 0.40	1.03 ± 0.38	1.11 ± 0.40	1.19 ± 0.51	1.14 ± 0.52	1.00 ± 0.41	1.03 ± 0.41	1.06 ± 0.37
$C_{\max}$ ($\mu\text{g/mL}$)	N/A	0.45 ± 0.25	0.26 ± 0.13	N/A	0.16 ± 0.11	0.027 ± 0.015	0.009 ± 0.002	0.41 ± 0.24	0.017 ± 0.006	0.011 ± 0.007	0.080 ± 0.057	0.049 ± 0.026
CL/F ($\text{L h}^{-1} \text{ kg}^{-1}$)	N/A	1.32 ± 0.58	1.77 ± 0.84	N/A	0.70 ± 0.44	2.16 ± 0.84	14.5 ± 0.6	0.82 ± 0.61	5.98 ± 2.56	2.75 ± 0.71	1.14 ± 0.69	1.30 ± 0.59

glucuronide conjugates of 6-, 8-, and 10-gingerols and 6-shogaol were 1.6, 1.0, 2.1, and 1.5 h, respectively.

Sulfate Conjugates of 6-, 8-, and 10-Gingerols and 6-Shogaol in the Plasma After Single Dose of Ginger Powder Capsules

The sulfate conjugates of 6-, 8-, and 10-gingerols and 6-shogaol were also determined after sulfatase hydrolysis. 6-Gingerol sulfate conjugate was detected from 0.25 to 8 h with peak concentration of $0.28 \pm 0.15 \mu\text{g/mL}$ at 1 h. 8-Gingerol sulfate conjugate was observed from 0.5 to 4 h with peak concentration of $0.28 \pm 0.18 \mu\text{g/mL}$ at 1 h. 10-Gingerol sulfate conjugate was observed from 0.25 to 10 h with peak concentration of $0.017 \pm 0.007 \mu\text{g/mL}$ at 1 h. 6-Shogaol sulfate conjugate was observed from 0.25 to 6 h with peak concentration of $0.047 \pm 0.035 \mu\text{g/mL}$ at 1 h (Fig. 4).

Similarly, to analyze the pharmacokinetic parameters of sulfate conjugates, the concentrations were calculated with the subtraction of free analyte concentrations. The pharmacokinetic parameters of the sulfate metabolites of 6-, 8-, and 10-gingerols and 6-shogaol were shown in Table IV. Their half-lives were 1.8, 1.3, 3.2, and 1.4 h, respectively.

Concentrations of 6-, 8-, and 10-Gingerols and 6-Shogaol and their Conjugate Metabolites in the Plasmas after Multiple Doses of Ginger Powder Capsules

A total of 23 healthy human subjects received either placebo ($n=11$) or ginger extracts 2.0 g/day ($n=12$) for 24 days. The blood samples were drawn within 24 h of the last dose. Free 6-, 8-, and 10-gingerols and 6-shogaol and their conjugate metabolites were determined. Due to their short half-lives, no free 6-, 8-, and 10-gingerols and 6-shogaol were detected in the plasma of all the subjects 24 h after the last dosing. These data suggest that there was no accumulation of free 6-, 8-, and 10-gingerols and 6-shogaol in plasma after multiple daily dosing. Low levels of 6-gingerol glucuronide (ranging from 5.43 to 13.6 ng/mL), 6-gingerol sulfate (ranging from 6.19 to 7.29 ng/mL), and 10-gingerol glucuronide (ranging from 6.96 to 9.33 ng/mL) were observed in four subjects who received ginger extracts. The levels of 10-gingerol sulfate, 8-gingerol glucuronide, 8-gingerol sulfate, 6-shogaol glucuronide, and 6-shogaol sulfate were below the detection limits in all the participants. These data demonstrated no accumulation of 6-, 8-, and 10-gingerols and 6-shogaol or their conjugate metabolites in plasma after 24 days of multiple daily dosing regimens due to their short half-lives and fast clearance.

Concentrations of 6-, 8-, and 10-Gingerols and 6-Shogaol and Their Conjugate Metabolites in the Colon Tissues After Multiple Doses of Ginger Powder Capsules in Normal- and High-Risk Colon Cancer Subjects

Although 6-gingerol was shown to have a high concentration in the gastrointestinal tract after p.o. dosing (31), no free 6-gingerol and its metabolites were detected in the colon tissues at 24 h after multiple daily dose ginger powder capsules. Only marginal levels of 10-gingerol glucuronide and sulfate conjugates were detected at 24 h after multiple

daily doses of ginger powder capsules which ranged from 1.72 to 2.76 ng/mL.

DISCUSSION

In a previous study, Wang *et al.* (3) developed a HPLC/MS method to quantify 6-, 8-, and 10-gingerols and 6-shogaol in rat plasma after oral administration of ginger oleoresin. The LLOQ ranged from 3.57 to 10.4 ng/mL for 6-, 8-, and 10-gingerols and 6-shogaol. Free 6-, 8-, and 10-gingerols and 6-shogaol were detected in rat plasma with varied concentrations at a dose of 300 mg/kg (p.o.). However, only the glucuronide conjugate of 6-gingerol was detected in rat plasma, whereas the levels of the glucuronide conjugates of 8- and 10-gingerols and 6-shogaol were under the LLOQ. Another study at a p.o. dose of 30 mg/kg of purified 6-gingerol in rats failed to detect free 6-gingerol in rat plasma (32).

In the current study, we developed a LC-MS/MS method for the simultaneous quantification of 6-, 8-, and 10-gingerols and 6-shogaol with LLOQ ranging from 2 to 5 ng/mL, which was more sensitive compared with the LC-MS method developed by Wang *et al.* In addition, we analyzed the pharmacokinetics of these four analytes and their glucuronide and sulfate metabolites in human plasma. Our data indicate that only free 10-gingerol and 6-shogaol were detected in the human plasma, whereas the majority of the 6-, 8-, and 10-gingerols and 6-shogaol existed as glucuronide and sulfate metabolites after oral dosing of 2 g ginger extracts. Furthermore, due to the short half-lives of the four analytes, no accumulation was observed in the plasma after multiple daily doses. Moreover, no analytes or their conjugates were detected in the colon tissues at 24 h after multiple dosing.

Our data suggest that both the glucuronide and sulfate conjugates of 6-, 8-, and 10-gingerols and 6-shogaol are present in human plasma. No free 6-gingerol was detected in plasma despite it being the most abundant component of ginger extracts (2.64%). In comparison, although 6-shogaol makes up 2.25% and 10-gingerol only accounts for 1.22% of the ginger extracts, 6-shogaol and 10-gingerol free compounds were detected in the human plasma. Conversion of 6-gingerol to 6-shogaol in gastrointestinal tract needs to be further explored as a previous report demonstrated that 6-gingerol and 6-shogaol inter-converted to each other in simulated gastric fluid (33). Overall, all the 6-gingerol, 8-gingerol, and the majority of 10-gingerol and 6-shogaol were present as either the glucuronide conjugate or sulfate conjugate, and only small amounts of 10-gingerol and 6-shogaol were present as the free drugs. Therefore, the pharmacological efficacy of 6-shogaol and 10-gingerol needs to be further validated. In fact, reports showed that 6-shogaol and 10-gingerol were much more pharmacologically active than 6-gingerol or 8-gingerol (19,29,34). However, the peak concentrations determined for 10-gingerol and 6-shogaol were 9.5 ± 2.2 ng/mL (0.027 ± 0.006 μ M) and 13.6 ± 6.9 ng/mL (0.049 ± 0.025 μ M), which were less than the IC₅₀'s of 10-gingerol and 6-shogaol (12 and 8 μ M, respectively). Although gingerols and shogaol might be higher in colon tissues (31), in the present study, only limited amounts of 10-gingerol glucuronide and sulfate conjugates were detected at 24 h after multiple daily dosing. To better understand the pharma-

cokinetics of gingerols and shogaol in colon tissues and correlate the pharmacokinetic to pharmacodynamic effect, early sampling time would be required to quantify the drug concentrations in colon tissues in future clinical studies.

In the present study, we measured 6-, 8-, and 10-gingerols, 6-shogaol, and their glucuronide and sulfate conjugates in human plasma and colon tissues. However, it is worth noting that other metabolites of gingerols and shogaol might also have pharmacological activity. For instance, *rac*-6-dihydroparadol, a mammalian metabolite of 6-gingerol and 6-shogaol which was chemically and metabolically stable, was shown to inhibit I κ B- α degradation and NF- κ B nuclear translocation, thus decreasing iNOS protein expression and suppressing NO synthesis in murine macrophage, which finally led to an anti-inflammatory effect (35). In addition, the other components of ginger powders or extracts such as paradols were not quantified due to the lack of commercially available standards. Paradols have shown pharmacological effects similar to gingerols and shogaols (36–39). All these components warrant future studies.

CONCLUSION

In conclusion, this study developed and validated a sensitive LC-MS/MS method for the simultaneous quantification of 6-, 8-, and 10-gingerols and 6-shogaol in the human plasma. Low levels of free 10-gingerol and 6-shogaol were detected in human plasma, whereas most of the 6-, 8-, and 10-gingerols and 6-shogaol existed in the form of glucuronide or sulfate conjugates. The pharmacokinetics of 6-, 8-, and 10-gingerols and 6-shogaol and their metabolites were analyzed. The half-lives of all compounds and their metabolites are between 1 and 3 h in human plasma.

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