

Pharmacokinetics of 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol and Conjugate Metabolites in Healthy Human Subjects

Suzanna M. Zick,¹ Zora Djuric,¹ Mack T. Ruffin,¹ Amie J. Litzinger,¹ Daniel P. Normolle,² Sara Alrawi,¹ Meihua Rose Feng,⁴ and Dean E. Brenner³

Departments of ¹Family Medicine, ²Radiation Oncology, and ³Internal Medicine; and ⁴College of Pharmacy and Engineering, University of Michigan Medical School, Ann Arbor, Michigan

Abstract

Background: Ginger shows promising anticancer properties. No research has examined the pharmacokinetics of the ginger constituents 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in humans. We conducted a clinical trial with 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol, examining the pharmacokinetics and tolerability of these analytes and their conjugate metabolites. **Methods:** Human volunteers were given ginger at doses from 100 mg to 2.0 g ($N = 27$), and blood samples were obtained at 15 minutes to 72 hours after a single p.o. dose. The participants were allocated in a dose-escalation manner starting with 100 mg. There was a total of three participants at each dose except for 1.0 g ($N = 6$) and 2.0 g ($N = 9$).

Results: No participant had detectable free 6-gingerol, 8-gingerol, 10-gingerol, or 6-shogaol, but 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol glucuronides were detected. The 6-gingerol sulfate conjugate was

detected above the 1.0-g dose, but there were no detectable 10-gingerol or 6-shogaol sulfates except for one participant with detectable 8-gingerol sulfate. The C_{max} and area under the curve values (mean \pm SE) estimated for the 2.0-g dose are 0.85 ± 0.43 , 0.23 ± 0.16 , 0.53 ± 0.40 , and 0.15 ± 0.12 $\mu\text{g/mL}$; and 65.633 ± 44.4 , 18.1 ± 20.3 , 50.1 ± 49.3 , and 10.9 ± 13.0 $\mu\text{g}\cdot\text{hr/mL}$ for 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol. The corresponding t_{max} values are 65.6 ± 44.4 , 73.1 ± 29.4 , 75.0 ± 27.8 , and 65.6 ± 22.6 minutes, and the analytes had elimination half-lives <2 hours. The 8-gingerol, 10-gingerol, and 6-shogaol conjugates were present as either glucuronide or sulfate conjugates, not as mixed conjugates, although 6-gingerol and 10-gingerol were an exception.

Conclusion: Six-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol are absorbed after p.o. dosing and can be detected as glucuronide and sulfate conjugates. (Cancer Epidemiol Biomarkers Prev 2008;17(8):1930–6)

Introduction

The ginger root (*Zingiber officinale* Roscoe, Zingiberaceae) is one of the most heavily consumed dietary substances in the world (1). Ginger was first cultivated in Asia (2) and has been used as a medicinal herb for at least 2,000 years (2). Medicinal references to ginger appear in early Sanskrit and Chinese texts as well as ancient Greek, Roman, and Arabic medical literature (3). In Western herbal medicine, ginger is used primarily as a remedy for digestive disorders, including dyspepsia, colic, nausea, vomiting, gastritis, and diarrhea (4). The dietary prevalence of foods, such as ginger, garlic, soy, curcumin, chilies, and green tea, are thought to contribute to the decreased incidence of colon, gastrointestinal, prostate, breast, and other cancers in Southeast Asian countries (5).

Ginger contains ~1.0% to 3.0% volatile oils and a number of pungent compounds (6). Gingerols are the

most abundant pungent compounds in fresh roots, and several gingerols of various chain lengths (n_6 to n_{10}) are present in ginger, with the most abundant being 6-gingerol. Shogaols, the dehydrated form of gingerols, are found only in small quantities in the fresh root and are mainly found in the dried and thermally treated roots, with 6-shogaol being the most abundant (7).

Studies in animal models have shown that ginger and its phenolic constituents (i.e., 6-gingerol) suppress carcinogenesis in the skin (1, 8–12), gastrointestinal tract (13), colon (14, 15), and breast (16). Ginger extracts have been tested for both antitumor promotion and apoptotic potential in several *in vitro* cell lines, including leukemia (17), and gastric (18), prostate (19), ovarian (20), and lung carcinoma (21). The chemopreventive mechanisms of ginger are not well understood but are thought to involve the up-regulation of carcinogen-detoxifying enzymes (22), and antioxidant (23–28) and anti-inflammatory (7, 29–31) activity. Ginger also inhibits nuclear factor- κ B activation induced by a variety of agents (10, 32–34) and has been shown to down-regulate nuclear factor- κ B-regulated gene products involved in cellular proliferation and angiogenesis, including interleukin 8 (19) and vascular endothelial growth factor (35). These factors have also been shown to promote tumor cell proliferation and angiogenesis and affect apoptotic response in several cancers.

Received 12/21/07; revised 4/11/08; accepted 5/13/08.

Grant support: University of Michigan General Clinical Research Center grant M01-RR00024; National Cancer Institute grants CN-55124, P30 CA48592, and K07CA102592; and National Center for Complementary and Alternative Medicine grant R21AT0001735.

Requests for reprints: Suzanna M. Zick, Suite 2E, 715 E. Huron, University of Michigan Medical Center, Ann Arbor, MI 48104. Phone: 734-998-7715; Fax: 734-998-7720. E-mail: szick@med.umich.edu

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doi:10.1158/1055-9965.EPI-07-2934

Only a handful of studies in rats have examined the absorption, bioavailability, metabolites, and elimination of ginger constituents (26, 36-39). Only two of the pungent compounds, 6-gingerol and zingerone, have been investigated, and, in two of these studies, 6-gingerol was administered as an i.v. bolus (36, 37), which is unlikely to be reflective of the usual p.o. dosing. No pharmacokinetic studies have been conducted in humans nor have any studies in mammals or *in vitro* examined the other major pungent constituents, namely 8-gingerol, 10-gingerol, and 6-shogaol.

I.v. bolus studies in rats indicated that the plasma concentration-time curve of 6-gingerol was illustrated by a two-compartment open model (36), and the serum protein binding of 6-gingerol was found to be >90% (36, 37). In both healthy normal rats and rats with acute renal failure, an i.v. bolus of 6-gingerol was rapidly cleared from the plasma with a terminal half-life ranging from 7.23 minutes to 8.5 minutes (36, 37). The terminal phase of 6-gingerol increased significantly to 11 minutes in rats with acute hepatic failure (37). More than 60% of a p.o. dose of 50-mg/kg dose of 6-gingerol was excreted as metabolites in the bile (48%) and urine (16%) within 60 hours (38). A 100-mg/kg p.o. dose of zingerone was found to have similar patterns of elimination of 6-gingerol, with 50% excreted in the feces and 40% excreted in the urine over 24 hours (26, 39).

When given p.o. in rats, 6-gingerol is readily conjugated in the intestinal epithelium and the liver to (S)-[6]-gingerol-4'-O- β -glucuronide and excreted through the bile (40). Six minor metabolites [vanillic acid, ferulic acid, (S)-(+)-hydroxy-6-oxo-8-(4-hydroxy-3-methoxyphenyl), octanoic acid, 4-(4-hydroxy-3-methoxyphenyl) butanoic acid, and 9-hydroxy[6]-gingerol] have also been detected in the urine (38).

Ginger and its constituents at doses up to 2.0 g daily have shown very low levels of toxicity and high levels of tolerability in both animals and humans, with only mild gastrointestinal complaints being reported (6). However, it is unclear if low levels of toxicity are due to poor p.o. bioavailability or a high degree of safety of pungent ginger constituents (i.e., gingerols and shogaols) in humans. This clinical trial evaluated the pharmacokinetic profile of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and their conjugate metabolites at 6 dose levels, 100, 250, 500, 1,000, 1,500, and 2,000 mg, administered p.o. to 27 healthy human volunteers. The purpose of this study was to: (a) determine if ginger extract standardized to 5% gingerols, in capsule formulation, is absorbed and biotransformed in humans; (b) assess the human pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and their conjugate metabolites; and (c) evaluate the safety and tolerability of up to 2.0 g of a single p.o. dose of ginger extract standardized to 5% gingerols.

Materials and Methods

The ginger product used in this study was manufactured by Pure Encapsulations[®]. The Pure Encapsulation[®] ginger (*Z. officinale*) powder is processed according to Good Manufacturing Practice guidelines. Each capsule contained 250 mg dry extract of ginger root [10:1 (volume

for volume) extraction solvent (50% ethanol) to root ratio] standardized to 15 mg (5%) of total gingerols. Based on high-performance liquid chromatography (HPLC) analysis, a 250-mg capsule of ginger extract contained 5.38 mg (2.15%) 6-gingerol, 1.80 mg (0.72%) 8-gingerol, 4.19 mg (1.78%) 10-gingerol, and 0.92 mg (0.37%) 6-shogaol. The gingerol content was verified by an independent laboratory with the use of the appropriate HPLC techniques (Integrated Biomolecule Corp.). The entire study was conducted with a single batch of ginger-powder extract to optimize product consistency. β -17-estradiol acetate and the enzymes 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. Six-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol were purchased from Chromadex. The standards were found to be >95% pure per HPLC analysis. Pelargonic acid vanillylamide, the internal standard, was obtained from Sigma and is >97% pure. Acetonitrile, methanol, hexane, and de-ionized water were all HPLC grade (Burdick & Jackson). HPLC-grade ethyl acetate and ammonium acetate were purchased from Fisher Scientific. HPLC-grade acetic acid was obtained from J.T. Baker.

Clinical Trial Design. Twenty-seven healthy volunteers were solicited by advertisement or word of mouth. The participants needed to be 18 y of age or older, in good health, and not taking any chronic medications. The participants were asked to avoid all foods containing ginger within the 14 d prior to drug administration and completed a food checklist to verify that they were not consuming any ginger-rich foods, such as ginger ale or Japanese food. This was a dose-escalation study and, as such, three participants were assigned per dose level, starting at the lowest dose of 100 mg and to each subsequent dose (250 mg, 500 mg, 1.0 g, 1.5 g, and 2.0 g) except for the 1.0-g ($N = 6$) and the 2.0-g ($N = 9$) doses. Six additional participants were assigned to the highest tolerated dose, 2.0 g, to ascertain toxicity, and three participants were added to the 1.0-g dose to act as training samples. After the administration of a single p.o. dose, blood was drawn from the participants at baseline and at 15, 30, and 45 min as well as at 1, 2, 4, 6, 10, 24, 48, and 72 hr after ingestion of the ginger. The plasma fraction was separated from the blood immediately and kept at -20°C until assayed. Toxicities were graded based on the National Cancer Institute Common Toxicity Criteria version 2.0 and monitored continuously for the first 10 hr and then 24, 48, and 72 hr after ginger administration. All the participants received meals standardized to fiber, calorie, and fat content throughout the first 24 hr of the study. All study procedures were administered at the University of Michigan General Clinical Research Center after the participant gave written informed consent. The study was approved by the University of Michigan Institutional Review Board.

Extraction of 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol from Plasma. Plasma samples (490 μL) were spiked with 10 μL of various concentrations of combined working standards and 10 μL of the internal standard, pelargonic acid vanillylamide (100 $\mu\text{g}/\text{mL}$).

The samples were diluted with water and extracted with 2.0 mL ethyl acetate/hexane (1:1, volume for volume). After centrifugation, the upper organic layer was removed into a glass vial and dried under a stream of argon. The samples were resuspended in 60 μ L of acetonitrile and 40 μ L of water. The samples were filtered and then placed into autosampler vials for HPLC quantification.

Enzymatic Hydrolysis of 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol Conjugates. The samples were also assayed for conjugates after incubating the plasma samples with the enzymes β -glucuronidase and sulfatase through the method of Asai et al. (41). For these assays, the plasma samples (500 μ L) were mixed with water (500 μ L) and the internal standard (10 μ L, 100 μ g/mL). The samples were then mixed with 50 μ L of β -glucuronidase (50 μ L, 446 units) in sodium-phosphate buffer (0.1 mol/L, pH 6.8) and 45 μ L of sulfatase (45 μ L, 51.5 units) in sodium-acetate buffer (0.1 mol/L, pH 5.0), and incubated at 37°C for 1 hr. The samples were then extracted through the extraction procedure given above. To determine the amount of glucuronide and sulfate conjugates in plasma, the samples were incubated separately with the β -glucuronidase and sulfatase enzymes prior to extraction.

Quantitation of 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol in Plasma. Reverse-phase HPLC was used to quantify 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in plasma. Chromatographic separation was accomplished with the use of a Phenomenex Luna 4.6 \times 250 mm, S-5 μ m, C18 column that was coupled with a Phenomenex 4.0 \times 20 mm, 5 μ , C18 guard column. The mobile phase consisted of 2% ammonium acetate at pH 4.5/59% acetonitrile/39% water (v/v/v; A) and 100% acetonitrile containing 20 mL of 1.0 mol/L ammonium acetate at pH 4.5 (98:2, volume for volume; B). The extracted sample was eluted on a gradient mobile phase starting from 100% A at zero time to 100% B in 15 min in a Waters #4 curve (concave) gradient and then to 100% A in 1 min. This was followed by 100% reagent B for 5 min and completed with a column wash of 100% reagent A for 10 min at a flow rate of 0.8 mL/min. The injection volume was 20 μ L, and detection was done with electrochemical detection at 600, 550, and 500 mV; UV detection was done at 282 nm.

Standard curves were constructed with the use of plasma spiked with 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol. Plasma samples with no detectable ginger analytes were spiked with varying amounts of a standard solution of the four analytes (0.10, 0.25, 0.5, 1.0, 2.5, and 5.0 μ g/mL). Each sample was analyzed in duplicate.

Analytical Assessment/Quality Control. Six-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, and the internal standard (pelargonic acid vanillylamide) were well resolved by HPLC. A linear relationship between 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol plasma concentration and response was found in the concentration ranges 0.1 μ g/mL to 5.0 μ g/mL. The intraday accuracy ranged from 91% to 128%, and the precision was \leq 11.7% for all four analytes; the interday accuracy ranged from 91% to 113%, and the precision was \leq 11.7% at three concentrations (5.0, 1.0, and 0.25 μ g/mL) for all four analytes. The interday coefficients of determination

(R^2) spanned from 0.9894 to 0.9992 for all four analytes and were observed over 4 d with gingerol-spiked and shogaol-spiked plasma. The lower limit of quantitation for this method was 0.1 μ g/mL, except for 10-gingerol, which was 0.25 μ g/mL. The extraction efficiency of all four analytes at 5.0, 1.0, and 0.25 μ g/mL concentration levels derived from area ratios ranged from 82.5% to 165.3%, with extraction efficiencies $>$ 100%. This may be due to variability in the chromatographic peak quantitation and in the standard curve variances, leading to experimental error. The intraday coefficients of variation for the high, medium, and low concentrations ranged from 1.5% to 10.7% for all four analytes. The lower limit of detection for all four analytes was no less than 75 ng/mL.

Results

Subjects and Toxicity. Twenty-seven healthy volunteers, 9 males and 22 females, with a mean age of 25.2 \pm 8.4 years (range, 19-61 years) were recruited from April through September 2005. Nearly one half ($N = 13$, 48.1%) of the participants were Caucasian; one third were Asian ($N = 9$, 33.3%); and more than 7% ($N = 2$) reported being of Hispanic ethnicity. All toxicities reported are shown in Table 1. No toxicities greater than the National Cancer Institute Common Toxicity Criteria (version 2.0) grade 1 were reported. The major treatment-associated toxicities were minor gastrointestinal upsets, including eructation, heartburn, and indigestion.

Detection of 6-Gingerol, 8-Gingerol, 10-Gingerol, and 6-Shogaol and Conjugate Metabolites in Plasma Samples. All plasma samples were analyzed both with and without incubation and deconjugating enzymes. No free 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol were detected in the plasma of any participants. Consequently, the subsequent results refer exclusively to 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol conjugates that were quantified after the treatment of samples with β -glucuronidase and sulfatase.

The pharmacokinetic parameters (C_{max} , T_{max} , and area under the curve) of the three highest doses of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol conjugates are presented in Table 2. The AUC was calculated by extrapolating to the last observed time point with measurable concentrations. The $t_{1/2}$ was only calculated for the 2.0-g dose for all conjugates. The 2.0-g dose was the only dose in which the decline of plasma concentration was consistent enough to allow for half-life estimation. The T_{max} ranged from 30 to 80 minutes for all four conjugates at all doses. Gingerol and shogaol conjugates were completely eliminated from the plasma at the 4-hour time point although one participant who received a 2.0-g dose still had detectable 6-gingerol conjugates at 8 hours after ingestion. Only 6-gingerol conjugates were detectable below the 1.0-g dose. The area under the curve for the 6-gingerol conjugates were 2.8 (\pm 2.5) and 5.3 (\pm 3.0) μ g/mL for the 250-mg and 500-mg dose, respectively. The C_{max} for the lower 6-gingerol doses were 0.3 (\pm 0.3) and 0.4 (\pm 0.23) μ g/mL.

The concentrations (mean \pm SD) of the three highest doses of 6-gingerol glucuronides and sulfates at T_{max} are presented in Table 3. Little to no conjugate metabolites

Table 1. Adverse events by category and dosage

Adverse event	100 mg n (%)	250 mg n (%)	500 mg n (%)	1,000 mg n (%)	1,500 mg n (%)	2,000 mg n (%)
Total	0 (0)	2 (66.6)	1 (33.3)	3 (50.0)	2 (66.6)	6 (66.6)
Fatigue*	0 (0)	1 (33.3)	0 (0)	1 (16.6)	1 (33.3)	0 (0)
Gastrointestinal symptoms [†]	0 (0)	0 (0)	1 (33.3)	1 (16.6)	2 (66.6)	4 (44.4)
Headache	0 (0)	1 (33.3)	0 (0)	1 (16.6)	1 (33.3)	1 (11.1)
Other [‡]	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.3)	1 (11.1)

NOTE: National Cancer Institute Common Toxicity Criteria v.2.0.

*Fatigue includes difficulty concentrating.

[†]Gastrointestinal symptoms include eructation, heartburn, and indigestion.

[‡]Other includes decreased heart rate and burning with urination.

were detected below the 1.0-g dose and are thus not presented in Table 3. No 8-gingerol, 10-gingerol, and 6-shogaol sulfates were detectable even at doses of 1.0 g and above. In addition, glucuronide metabolites were negligible for 8-gingerol, 10-gingerol, and 6-shogaol except for the 2.0-g dose, in which $0.30 \pm 0.33 \mu\text{g/mL}$ of 8-gingerol, $0.18 \pm 0.26 \mu\text{g/mL}$ of 10-gingerol, and $0.14 \pm 0.25 \mu\text{g/mL}$ of 6-shogaol were detectable. The concentrations of conjugate metabolites were similar between the separate and mixed assays at all three dose levels (1.0, 1.5, and 2.0 g) for 8-gingerol, 10-gingerol, and 6-shogaol, except for 10-gingerol, in which there were more mixed conjugates compared with separate conjugates. For instance, at the 2.0-g dose, there was $0.18 \pm 0.20 \mu\text{g/mL}$ for the separate assay versus $0.36 \pm 0.26 \mu\text{g/mL}$ for the mixed assay.

The sulfate conjugates were below the detectable assay limits for all participants at any dose for both 10-gingerol and 6-shogaol, and only one participant at the 2.0-g dose for 8-gingerol had detectable sulfate conjugates. Six-gingerol sulfate conjugates were not detectable below

the 1.0-g dose, with only one third of the participants at 1.0 g and two thirds at the 1.5-g and 2.0-g dose having detectable concentrations. The glucuronide conjugates were similar, with only a few participants having detectable concentrations of any metabolites below 1.0 g of ginger.

The relative proportion of glucuronides to sulfates is presented in Table 3. The relative amount of glucuronide conjugates was higher than the amount of sulfate conjugates at each participant's observed T_{max} by 5% to 93% for 6-gingerol, 71% to 100% for 8-gingerol, 4% to 67% for 10-gingerol, and 0% to 100% for 6-shogaol.

Discussion

The results indicate that no free 6-gingerol, 8-gingerol, 10-gingerol, or 6-shogaol was detectable in the plasma within the dose range investigated. All four analytes were, however, quickly absorbed after p.o. dosing and can be detected as glucuronide and sulfate conjugates

Table 2. Six-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol pharmacokinetic parameters estimated from the raw data

6-gingerol	Dose = 1,000 mg (N = 6)		Dose = 1,500 mg (N = 3)		Dose = 2,000 mg (N = 8)	
	Mean \pm SD	Min (max)	Mean \pm SD	Min (max)	Mean \pm SD	Min (max)
AUC*	12.6 (6.4)	3.6 (20.5)	75.6 (110.3)	8.6 (203.0)	65.6 (44.4)	10.5 (162.6)
C_{max} *	0.4 (0.2)	0.2 (0.7)	1.69 (2.31)	0.20 (4.36)	0.85 (0.43)	0.19 (1.56)
$t_{1/2}$ [†]					110.0 (34.9)	86.6 (186.0)
t_{max}	55.0 (7.7)	45.0 (60.0)	60.0 (0.0)	60.0 (60.0)	65.6 (22.6)	45.0 (120.0)
8-gingerol						
AUC	2.1 (2.2)	0 (4.5)	2.6 (2.0)	1.1 (4.9)	18.1 (20.3)	2.8 (65.2)
C_{max}	0.1 (0.1)	0 (0.2)	0.1 (0.1)	0.03 (0.13)	0.23 (0.16)	0.06 (0.50)
$t_{1/2}$ [†]					113.5 (41.1)	90.0 (180.0)
t_{max}	52.5 (8.7)	45.0 (60.0)	60.0 (0.0)	60.0 (60.0)	73.1 (29.4)	45.0 (120.0)
10-gingerol						
AUC	2.9 (3.2)	0 (7.9)	7.7 (5.3)	3.7 (13.7)	50.1 (49.3)	4.2 (156.9)
C_{max}	0.1 (0.1)	0 (0.4)	0.1 (0.02)	0.08 (0.12)	0.53 (0.4)	0.11 (1.33)
$t_{1/2}$ [†]					128.7 (38.8)	90.0 (182.7)
t_{max}	60.0 (0.0)	60.0 (60.0)	80.0 (34.6)	60.0 (120.0)	75.0 (27.8)	60.0 (120.0)
6-shogaol						
AUC	0.8 (1.5)	0 (3.7)	1.6 (2.8)	0 (4.9)	10.9 (13.0)	1.7 (40.3)
C_{max}	0.1 (0.1)	0 (0.1)	0.04 (0.08)	0 (0.13)	0.15 (0.12)	0.02 (0.34)
$t_{1/2}$ [†]					120.4 (42.0)	85.9 (180.0)
t_{max}	55.0 (8.7)	45.0 (60.0)	60.0 (0.0)	60.0 (60.0)	65.6 (22.6)	45.0 (120.0)

NOTE: AUC was determined by the trapezoid rule; $t_{1/2}$ was determined by means of linear interpolation.

*AUC and C_{max} are recorded as $\mu\text{g/mL}$.

[†] $t_{1/2}$ and t_{max} are recorded in minutes.

[‡]These results are based on detectable free gingerols and shogaols after incubation with β -glucuronidase and sulfatase and thus represent combined conjugates.

Table 3. The fraction of 6-gingerol glucuronide and sulfate in plasma at C_{max} after administration of 1,000, 1,500, and 2,000 mg ginger extract based on enzymatic hydrolysis done in duplicate

Dose (mg)	6-gingerol glucuronide (µg/mL)	6-gingerol sulfate (µg/mL)	Total 6-gingerol	
			Separate assay (µg/mL)	Mixed assay (µg/mL)
1,000	0.16 ± 0.15	0.02 ± 0.03	0.18 ± 0.12	0.40 ± 0.20
1,500	0.62 ± 0.62	0.04 ± 0.04	0.66 ± 0.51	1.03 ± 0.40
2,000	0.62 ± 0.56	0.33 ± 0.41	0.95 ± 0.41	0.91 ± 0.50

NOTE: Total 6-gingerols in plasma were assayed after incubating the plasma with the enzymes β-glucuronidase and sulfatase separately (separate assay) and combined (mixed assay).

in the serum. The majority of the metabolites were glucuronide conjugates. No sulfate conjugates were detected, with the exception of 6-gingerol, in which only at the highest dose was any significant amount of sulfates detected and roughly on third of the conjugates were sulfates. Further, the metabolites seemed to be either glucuronides or sulfates and not mixed conjugates although, once again, 6-gingerol seemed to be an exception with more mixed conjugates seen at lower doses. These results are similar with those observed with p.o. dosing of 6-gingerol in rats, in which no free 6-gingerol was found at any time point in the urine or bile, but (S)-[6]-gingerol-4-O-β-glucuronide was present in the bile, and no sulfate conjugates were detectable (38). Further, Metzler and colleagues found that human intestinal microsomes and hepatic microsomes fortified with UDP-glucuronyl transferase enzymes only formed glucuronides of 6-gingerol and that UGT1A1, UGT1A3, and UGT2B7 (these are expressed in both the liver and intestinal mucosa) were responsible for the production of the glucuronides (40). Currently, however, it is unclear if gingerols/shogaols are conjugated to glucuronides in the intestinal mucosa, liver or both, and if free or already conjugated gingerols and shogaols reach the liver and are further conjugated with sulfate to form glucuronide/sulfate conjugates there. Future research will need to be conducted to determine the relative importance of UDP-glucuronyl transferase activity in the liver compared with that in the intestinal mucosa as well as the contribution of liver sulfate enzymes.

Ginger conjugates began to appear 30 minutes after p.o. dosing, reaching their T_{max} between 45 minutes to 120 minutes, with elimination half-lives ranging from 75 minutes to 120 minutes at the 2.0-g dose. These results differ from both the i.v. bolus and p.o. studies conducted in rats. I.v. bolus studies in rats found that free 6-gingerol is rapidly cleared from the plasma with a terminal half-life ranging from 7.23 minutes to 8.5 minutes and that 6-gingerol is not detectable after 30 minutes (36). Whereas a single p.o. dosing of 6-gingerol in rats resulted in the rapid appearance of glucuronide conjugate, it did not reach its maximum concentration until after 12 hours and was detectable for at least 60 hours after ingestion (38). The difference between the present study and the i.v. injection of 6-gingerol would seem to be primarily due to the method of delivery. I.v. 6-gingerol would bypass being metabolized by the gut bacteria, the intestinal epithelium, or the liver and, thus, in its nonconjugated form that is detectable quickly after injection. Also, it seems that i.v. free 6-gingerol was much more rapidly

cleared from the system compared with conjugate metabolites. The difference in time to maximum concentration and elimination between the p.o.-administered 6-gingerol in rats and in humans could be due to differences between species or differences in dose. The dose of 6-gingerol given to the rats was approximately equivalent to a human dose of 583.3 mg of 6-gingerol. This is much higher than 43.04 mg, the maximum amount of 6-gingerol the participants in our study were given.

The maximum serum concentrations of the ginger analytes were reached at either the 1.5-g or 2.0-g dose and were 1.69 µg/mL for 6-gingerol, 0.23 µg/mL for 8-gingerol, 0.53 µg/mL for 10-gingerol, and 0.15 µg/mL for 6-shogaol. The lack of free gingerols and shogaol and the low concentration of ginger metabolites in the serum can be used to assess the potential clinical relevance of the reported *in vitro* research employing these ginger components. For instance, in prostate cancer cell lines, 14.72 µg/mL (50 µM) of 6-gingerol was needed to inhibit MKP5, a key mediator of pro-inflammatory pathways and cancer cell growth in prostate cells (19). In another study, 6-shogaol was found to induce apoptosis, autophagocytosis, and growth inhibition in ovarian cancer cells at 2.21 µg/mL (7.5 µmol/L; ref. 20). All of these *in vitro* studies required higher concentrations of free ginger constituents than those found in the serum in this study, putting the clinical validity of these and similar studies in question. However, gingerols and shogaols may reach higher serum concentrations in target tissue compared with the serum (e.g., gut). Ginger conjugates may also be as or more biologically active compared with parent compound (40). Clearly, further research is needed to answer these questions and determine the cancer prevention relevance of ginger.

In this trial, no serious adverse effects were reported after ingesting up to 2.0 g of standardized ginger extract. All toxicities reported were mild and correspond to grade 1 of the National Cancer Institute common toxicity scale (Table 1). Consistent with previous clinical research, the majority of the adverse events were transient gastrointestinal upsets, such as gas and bloating. Whereas the small size of this trial precludes any formal safety endpoint analysis and statistical certainty of safety, the safety profile observed here is consistent with previous clinical and preclinical data (6).

With the exception of 6-gingerol, the analytes were not well absorbed, with no detectable conjugate metabolites below the 1.0-g ginger-extract dose. The lack of detectable analytes below the 1.0-g dose was likely due to the low amount of individual analytes in the ginger extract,

with only 21.52 mg of 6-gingerol, 7.20 mg of 8-gingerol, 16.76 mg of 10-gingerol, and 3.68 mg of 6-shogaol in the 1.0-g dose. Another possible reason for the lack of detectable analytes could be the lack of stability of gingerols and shogaols in plasma during storage and analysis. This explanation seems unlikely because 6-gingerols seem stable in conditions similar to the ones used in our analysis although the stability of 6-gingerols was only determined in aqueous solution and not in plasma (42). Because of low levels of absorption, the participants receiving the highest dose did not have adequate detectable concentrations after C_{max} to reliably calculate the elimination half-life. Consequently, no pharmacokinetic model was able to be constructed, and the pharmacokinetic parameters are based on noncompartment analysis with an elimination half-life only presented for the 2.0-g dose. Even at the 2.0-g dose, the half-life needs to be interpreted with caution because estimates were based on a limited data set.

Future studies should focus on obtaining information for conducting both single-dose and multi-dose pharmacokinetic modeling. The information gained from pharmacokinetic modeling could then be used to optimize the dose and dose regimen in clinical phase-2 or phase-3 trials and to enhance the delivery of the ginger extract. Pharmacokinetic models could be developed by enriching the number of serum samples taken between baseline and 4 hours after p.o. administration, with particular emphasis on the serum concentrations after 60 minutes when, on average, time to maximum concentration is reached. In addition, higher doses of pungent gingerol constituents could be administered, or more sensitive analytic methods for detecting gingerol and shogaol conjugates in the picogram concentration could be developed. All three of these techniques would help to better describe pharmacokinetic parameters and ensure the calculation of accurate elimination half-lives. Multi-dose pharmacokinetic parameters and safety also need to be investigated to explore the possibility of any toxicity or tolerability issues from longer-term dosing. Pharmacokinetic information studies in populations that are likely to be the target for ginger therapeutically, such as people at high risk for colorectal cancer and older populations with common comorbidities, also need to be conducted. Further work is also needed to determine the range of gingerol and shogaol metabolites found in humans and the activity of these metabolites to modulate important cancer markers, such as $Nf\kappa\beta$ or a variety of inflammatory eicosanoids (i.e., prostaglandin E_2).

In summary, the main pungent constituents of ginger root, 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol, are quickly absorbed and detected in the serum as glucuronide and sulfate conjugates, with the majority detected as glucuronide metabolites. These constituents, at concentrations normally found in ginger root (0.5%-2.5%), are detectable in the serum starting at a 1.0-g dose with the exception of 6-gingerol, which is detectable at a 250-mg dose with maximum concentrations ranging from 0.1 $\mu\text{g}/\text{mL}$ to 1.7 $\mu\text{g}/\text{mL}$.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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We thank Pure Encapsulations® (Sudbury, Massachusetts) for donating the ginger extract.

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Cancer Epidemiol Biomarkers Prev 2008;17:1930-1936.

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