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

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Abstract

Background—Ginger demonstrates promising anticancer properties. No research has examined the pharmacokinetics of the ginger constituents 6-, 8-, 10-gingerol and 6-shogaol in humans. We conducted a clinical trial with 6-, 8-, 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 oral dose. 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-, 8-, 10-gingerol or 6-shogaol, but 6-, 8-, 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 Cmax and AUC values (Mean±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 μg/mL ; and 65.6.33±44.4, 18.1±20.3, 50.1 ±49.3, and 10.9±13.0 μg·hr/mL for 6-, 8-, 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 < 2hr. The 8-, 10-gingerol and 6-shogaol conjugates were present as either glucuronide or sulfate conjugates, not as mixed conjugates, although 6-, 10-gingerol were an exception.

Conclusion—Six-, 8-, 10-gingerol and 6-shogaol is absorbed after oral dosing and can be detected as glucuronide and sulfate conjugates.

Keywords

Chemoprevention; Pharmacokinetics; Gingerols; Shogaol; Ginger; Metabolism; Clinical trial

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

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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] 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 South East Asian countries.[5]

Ginger contains approximately 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 (n6 to n10) are present in ginger, with the most abundant being 6-gingerol. Shogaols, the dehydrated form of gingerols, are found in only 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 skin,[8,1,9–12] gastrointestinal tract,[13] colon.[14,15] and breast..[16] Ginger extracts have been tested for both anti-tumor promotion and apoptotic potential in several *in vitro* cell lines, including leukemia,[17] gastric,[18] prostate,[19] ovarian [20] and lung carcinoma.[21] Ginger's chemopreventive mechanisms are not well understood but are thought to involve up-regulation of carcinogen detoxifying enzymes,[22] anti-oxidant [23–28] and anti-inflammatory [29–31,7] activity. Ginger also inhibits NF-κB activation induced by a variety of agents,[32,33,10,34] and has been shown to down regulate NF-κB regulated gene products involved in cellular proliferation and angiogenesis, including IL-8,[19] and VEGF.[35] These factors have also been shown to promote tumor cell proliferation, angiogenesis, and affect apoptotic response in several cancers.

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 intravenous bolus,[36,37] which is unlikely to be reflective of usual oral 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- and 10-gingerols and 6-shogaols.

Intravenous bolus studies in rats indicated that the plasma concentration time curve of 6-gingerol was illustrated by a two-compartment open model,[36] and serum protein binding of 6-gingerol was found to be greater than 90%.[36,37] In both healthy, normal rats and rats with acute renal failure an intravenous bolus of 6-gingerol was rapidly cleared from plasma with a terminal half-life ranging from 7.23 to 8.5 minutes.[36,37] The terminal phase of 6-gingerol increased significantly to 11 minutes in rats with acute hepatic failure.[37] Over 60% of an oral 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 oral dose of Zingerone was found to have similar patterns of elimination to 6-gingerol with 50% excreted in the feces and 40% in the urine over 24 hours.[39,26].

When given orally 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.[41]. 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, 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 demonstrated 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 oral 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-, 8-, and 10-gingerol and 6-shogaol and their conjugate metabolites at six dose levels: 100, 250, 500, 1000, 1500, 2000 mg administered orally to twenty-seven healthy human volunteers. The purpose of this study was to: 1) determine if a ginger extract standardized to 5% gingerols, in capsule formulation, is absorbed and bio-transformed in humans; 2) assess the human pharmacokinetics of 6-, 8-, and 10-gingerol and 6-shogaol and their conjugate metabolites; and 3) evaluate the safety and tolerability of up to 2.0 g of a single-oral dose of a ginger extract standardized to 5% gingerols.

Materials and Methods

The ginger product used in this study was manufactured by Pure Encapsulations® (Sudbury, MA). Pure Encapsulation's® ginger (*Z. officinale*) powder is processed using Good Manufacturing Procedures (GMP). Each capsule contained 250 mg dry extract of ginger root [10:1 (v/v) extraction solvent (ethanol 50 %):root] standardized to 15 mg (5%) of total gingerols. Based on 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. Gingerol content was verified by an independent laboratory using appropriate HPLC techniques (Integrated Biomolocule Corporation: Tucson, Arizona). The entire study was conducted using a single batch of ginger powder extract to optimize product consistency. β-17-estradiol acetate, and the enzymes β-glucuronidase (Type IX-A from E.coli) and sulfatase (Type H-1 from Helix pomatia), were purchased from Sigma- Aldrich Inc (St. Louis, MO). Sodium phosphate and sodium acetate (ACS certified) were purchased from Fisher Scientific (Fair Lawn, NJ). Six, 8, and 10-gingerols and 6-shogaol were purchased from Chromadex (Santa Ana, CA, USA) (Fig. 1). Standards were found to be >95% pure per HPLC analysis. Pelargonic acid vanillylamide (PAV), the internal standard, was obtained from Sigma (St. Louis, MO, USA) and is ≥ 97% pure. Acetonitrile, methanol, hexane and de-ionized water were all HPLC grade (Burdick & Jackson, Muskegon, MI, USA). HPLC grade ethyl acetate and ammonium acetate were purchased from Fisher Scientific (Pittsburgh, PA, USA). HPLC grade acetic acid was obtained from J.T. Baker (Phillipsburg, NJ, USA).

Clinical Trial Design

Twenty-seven healthy volunteers were solicited by advertisement or word of mouth. Participants needed to be 18 years of age or older, in good health and not taking any chronic medications. 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 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 oral dose, blood was drawn from the participants at baseline, 15, 30, and 45 minutes as well as at 1, 2, 4, 6, 10, 24, 48 and 72 hours after ingestion of the ginger. The plasma fraction was separated from blood immediately, and kept at −20°C until assayed. Toxicities were graded based on National Cancer Institute Common Toxicity Criteria version 2.0 and monitored continuously for the first 10 hours and then 24, 48 and 72 hours after ginger administration. All participants received meals standardized to fiber, calorie, and fat content throughout the first 24 hours of the study. All study procedures were administered at the University of Michigan General Clinical Research Center (GCRC) after the participant gave written, informed consent. The study was approved by the University of Michigan Institutional Review Board.

Extraction of 6-, 8-, and 10-Gingerols 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 internal standard, PAV (100 μg/mL). The samples were diluted with water and extracted with 2.0 mL ethyl acetate:hexane (1:1 v/v). After centrifugation the upper organic layer was removed into a glass vial and dried under a stream of argon. The samples were re-suspended in 60 μL of acetonitrile and 40 μL of water. Samples were filtered and then placed into autosampler vials for HPLC quantification.

Enzymatic Hydrolysis of 6-, 8-, and 10-Gingerols and 6-Shogaol Conjugates

Samples were also assayed for conjugates, after incubating the plasma samples with the enzymes β-glucuronidase and sulfatase using the method of Asai et al.[40] For these assays, plasma samples (500 μL) were mixed with water (500 μL), and internal standard (10 μL, 100 μg/mL). The samples were then mixed with 50μL of β-glucuronidase (50 μL, 446 units) in sodium phosphate buffer (0.1 M, pH6.8) and 45μL of sulfatase (45 μL, 51.5 units) in sodium acetate buffer (0.1 M, pH5.0), and incubated at 37°C for 1 hour. The samples were then extracted using the extraction procedure given above. To determine the amount of glucuronide and sulfate conjugates in plasma, samples were incubated separately with β-glucuronidase and sulfatase enzymes prior to extraction.

Quantitation of 6-, 8-, and 10-Gingerols and 6-Shogaol in Plasma

Reverse-phase HPLC was used to quantify 6-, 8-, and 10-gingerols and 6-shogaol in plasma. Chromatographic separation was accomplished using a Phenomenex Luna 4.6 mm × 250 mm, S-5 μm, C18 column that was coupled with a Phenomenex 4.0 × 20 mm, 5μ C18 guard column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of 2 % ammonium acetate at pH 4.5/59% acetonitrole/39% water (v/v/v; A) and 100% acetonitrile containing 20 mL of 1.0M ammonium acetate at pH 4.5 (98:2 v/v) (B). The extracted sample was eluted on a gradient mobile phase starting 100% of 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 minutes and completed with a column wash of 100% reagent A for 10 minutes at a flow rate of 0.8 mL/min. The injection volume was 20 μL and detection was performed using electrochemical detection (EC) at 600, 550 and 500 mV and ultraviolet (UV) detection at 282 nm.

Standard curves were constructed using plasma spiked with 6-, 8-, and 10-gingerols 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 μg/mL). Each sample was analyzed in duplicate.

Analytical Assessment/Quality Control

Six-, 8-, and 10-gingerols, 6-shogaol and internal standard (PAV) were well resolved by HPLC. A linear relationship between 6-, 8-, and 10-gingerols and 6-shogaol plasma concentration and response was found in the concentration ranges 0.1 to 5.0 μg/mL. The intra-day accuracy ranged from 91 to 128% and precision was ≤11.7% for all four analytes, and the inter-day accuracy ranged from 91–113% and the precision was ≤11.7% at three concentrations (5.0, 1.0, 0.25 μg/mL) for all four analytes. Inter-day coefficients of determination (R^2) spanned from 0.9894 to 0.9992 for all four analytes and were observed over four days with gingerol and shogaol spiked plasma. The lower limit of quantitation for this method was 0.1 μg/mL except for 10-gingerol which was 0.25 μg/mL. The extraction efficiency of all four analytes at 5.0, 1.0, 0.25 μg/mL concentrations levels derived from area ratios ranged from 82.5 to 165.3 % with extraction efficiencies greater than 100%, may be due to variability in chromatographic

peak quantitation and in standard curve variances leading to experimental error. The intra-day coefficients of variation (CV) 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, nine males and twenty-two females, mean age 25.2 ±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 over 7% (N=2) reported being of Hispanic ethnicity. All toxicities reported are shown in Table 1. No toxicities greater than NCI Common Toxicity Criteria (v. 2.0) Grade 1 were reported. The major treatment associated toxicities were minor gastrointestinal upsets including eructation, heartburn, and indigestion.

Detection of 6-, 8-, and 10-Gingerols 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-, 8-, 10-gingerols, and 6-shogaol were detected in the plasma of any participants. Consequently, subsequent results refer exclusively to 6-, 8-, 10-gingerols, and 6-shogaol conjugates that were quantified after treatment of samples with β-glucuronidase and sulfatase.

Pharmacokinetic parameters (C_{max}, T_{max} and AUC) of the three highest doses of 6-, 8-, 10-gingerols, 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 where 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 AUC for the 6-gingerol conjugates were 2.8 (± 2.5) and 5.3(± 3.0) μg/mL for the 250 and 500 mg dose respectively. The C_{max} for the lower 6-gingerol doses were 0.3 (± 0.3) and 0.4 (± 0.23) μg/mL.

The concentrations (mean ±SD) of the three highest doses of 6-gingerol glucoronides and sulfates at T_{max} are presented in (Table 3). Little to no conjugate metabolites were detected below the 1.0 g dose and are thus not presented in Table 3. No 8-, 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-, 10-gingerol and 6-shogaol except for at the 2.0g dose where 0.30 ± 0.33 μg/mL of 8-gingerol, 0.18 ± 0.26 μg/mL of 10-gingerol and 0.14 ± 0.25 μg/mL of 6-shogaol were detectable. Concentrations of conjugate metabolites were similar between the separate and mixed assays at all three dose levels (1.0, 1.5, 2.0 g) for 8-, 10-gingerol and 6-shogaol except for 10-gingerol where there was more mixed conjugates compared to separate conjugates. For instance, at the 2.0 g dose there was 0.18 ± 0.20 μg/mL for the separate assay versus 0.36 ± 0.26 μg/mL for the mixed assay.

Sulfate conjugates were below 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 and only 1/3 of participants at 1.0 g and 2/3 at the 1.5 and 2.0 g dose having detectable concentrations. 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-, 8-, 10-gingerol or 6-shogaol were detectable in the plasma within the dose range investigated. All four analytes were, however, quickly absorbed after oral dosing and can be detected as glucuronide and sulfate conjugates in serum. The majority of the metabolites were glucuronide conjugates. No sulfate conjugates were detected with the exception of 6-gingerol where only at the highest dose was any significant amount of sulfates detected, where roughly 1/3 of the conjugates were sulfates. Further, the metabolites appeared to be either glucuronides or sulfates and not mixed conjugates, although once again 6-gingerol appeared to be an exception with more mixed conjugates seen at lower doses. These results are similar with those observed with oral dosing of 6-gingerol in rats where 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 (UDTA) only formed glucuronides of 6-gingerol and that UGT1A1, 1A3 and UGT2B7 (these are expressed in both the liver and intestinal mucosa) were responsible for production of the glucuronides. [41] 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 as compared to that in the intestinal mucosa as well as the contribution of liver sulfate enzymes.

Ginger conjugates began to appear 30 minutes after oral dosing, reaching their T_{max} between 45 to 120 minutes, with elimination half-lives ranging from 75 to 120 minutes at the 2.0 g dose. These results differ from both the intravenous bolus and oral studies conducted in rats. Intravenous bolus studies in rats found that free 6-gingerol is rapidly cleared from plasma with a terminal half-life ranging from 7.23 to 8.5 minutes and that 6-gingerol is not detectable after 30 minutes.[36] While a single oral dosing of 6-gingerol in rats resulted in 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 intravenous injection of 6-gingerol would appear to be primarily due to the method of delivery. Intravenous 6-gingerol would bypass being metabolized by the gut bacteria, the intestinal epithelium or the liver and thus in its non-conjugated form that is detectable quickly after injection. Also, it appears that intravenous free 6-gingerol was much more rapidly cleared from the system compared to conjugate metabolites. The difference in time to maximum concentration and elimination between the orally administered 6-gingerol in rats and 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 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).[20] All of these *in vitro* studies required higher concentrations of free ginger constituents than 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 with in target tissue compared to serum, e.g., gut. Ginger conjugates may also be as or more biologically active compared to parent compound. [41] 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 NCI common toxicity scale (Table 1). Consistent with previous clinical research the majority of the adverse events were transient GI upsets such as gas and bloating. While 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. 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 lack of stability of gingerols and shogaols in plasma during storage and analysis. This explanation appears unlikely as 6-gingerols appear stable in conditions similar to the ones used in our analysis, although the stability of 6-ginerols was only determined in aqueous solution and not in plasma. [42] Because of low levels of absorption, 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 pharmacokinetic parameters are based on non-compartment 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 as 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 II/III 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 oral 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 analytical 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 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 Nfκβ or a variety of inflammatory ecosanoids, i.e., prostaglandin E_2.

In summary, the main pungent constituents of ginger root, 6-, 8-, 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 to 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 to 1.7 μg/mL.

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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)
GI 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)

*Fatigue includes: Difficulty Concentrating

†GI Symptoms include: Eructation, Heartburn, and Indigestion

‡Other includes: Decreased Heart Rate and Burning with Urination

**National Cancer Institute, Common Toxicity Criteria v.2.0

Table 2

6-, 8-, 10-gingerol and 6-shogaol pharmacokinetic parameters estimated from the raw data. AUC was determined by the trapezoid rule, and $t_{1/2}$ by means of linear interpolation

	Dose = 1000 mg (N = 6)		Dose = 1500 mg (N = 3)		Dose = 2000 mg (N = 8)	
6-Gingerol	**Mean±S.D.**	**(Min, Max)**	**Mean±S.D.**	**(Min, Max)**	**Mean±S.D.**	**(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)

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

† $T_{½}$ and t_{max} is recorded in minutes

§ C_{max} and AUCare recorded as μg/mL

Table 3

The fraction of 6-gingerol glucuronide and sulfate in plasma at C_{max} after administration of 1000, 1500 and 2000 mg ginger extract based on enzymatic hydrolysis performed in duplicate. Total 6-gingerols in plasma were assayed after incubating plasma with enzymes β-glucuronidase and sulfatase separately (Separate Assay) and combined (Mixed Assay).

			Total 6-Gingerol	
Dose (mg)	**6-gingerol glucuronide (μg/mL)**	**6-gingerol sulfate (μg/mL)**	**Separate Assay (μg/mL)**	**Mixed Assay (μg/mL)**
1000	0.16±0.15	0.02±0.03	0.18±0.12	0.40±0.20
1500	0.62±0.62	0.04±0.04	0.66±0.51	1.03±0.40
2000	0.62±0.56	0.33±0.41	0.95±0.41	0.91±0.50