BRIEF REPORT

Impact of Green Tea Catechin Ingestion on the Pharmacokinetics of Lisinopril in Healthy Volunteers

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Lisinopril, a highly hydrophilic long-acting angiotensin-converting enzyme inhibitor, is frequently prescribed for the treatment of hypertension and congestive heart failure. Green tea consumption may reduce the risk of cardiovascular outcomes and total mortality, whereas green tea or its catechin components has been reported to decrease plasma concentrations of a hydrophilic β blocker, nadolol, in humans. The aim of this study was to evaluate possible effects of green tea extract (GTE) on the lisinopril pharmacokinetics. In an open-label, randomized, single-center, 2-phase crossover study, 10 healthy subjects ingested 200 mL of an aqueous solution of GTE containing ~ 300 mg of (–)-epigallocatechin gallate, a major catechin component in green tea, or water (control) when receiving 10 mg of lisinopril after overnight fasting. The geometric mean ratio (GTE/control) for maximum plasma concentration and the area under the plasma concentration-time curve of lisinopril were 0.289 (90% confidence interval (CI) 0.226–0.352) and 0.337 (90% CI 0.269–0.405), respectively. In contrast, there were no significant differences in time to reach maximum lisinopril concentration (6 hours in both phases) and renal clearance of lisinopril (57.7 mL/minute in control vs. 56.9 mL/minute in GTE). These results suggest that the extent of intestinal absorption of lisinopril was significantly impaired in the presence of GTE, whereas it had no major effect on the absorption rate and renal excretion of lisinopril. Concomitant use of lisinopril and green tea may decrease oral exposure to lisinopril, and therefore result in reduced therapeutic efficacy.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Lisinopril, the most hydrophilic, long-acting angiotensin-converting enzyme inhibitor, is commonly used for the treatment for essential hypertension and congestive heart failure. Green tea consumption has been reported to be associated with reduced risks of cardiovascular outcomes and total mortality.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This study addressed whether the pharmacokinetics of lisinopril is influenced when orally administered with an aqueous solution of green tea extract (GTE).

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Plasma concentrations of lisinopril is significantly decreased when administered with an aqueous solution of GTE, containing ~ 300 mg of (–)-epigallocatechin gallate. There is no difference in renal clearance of lisinopril between those co-administered water and those co-administered GTE.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ These results suggest that green tea and its catechin supplement could reduce oral bioavailability of lisinopril, resulting in therapeutic inefficiency. Consumption of green tea products should be avoided during treatment with lisinopril.

Lisinopril is used in the treatment of hypertension and congestive heart failure, and is one of the most prescribed drugs in the United States in recent years.[1] Regarding physicochemical and pharmacokinetic properties, lisinopril, a carboxyalkyl dipeptide, is characterized by high solubility, poor membrane permeability, and negligible metabolism, and therefore it is excreted unchanged into urine after oral administration.[2,3] In addition, considering that the oral bioavailability of lisinopril is 29% despite its low permeability,[4] a carrier-mediated transport process may be required for the intestinal absorption and tissue distribution of lisinopril. Previous *in vitro* studies suggested that H$^+$/peptide transporter (PEPT1), which is expressed in the apical membrane of enterocytes, may contribute to cellular uptake of some angiotensin converting enzyme (ACE) inhibitors, such as fosinopril and zofenopril, whereas lisinopril has been found to be a poor substrate of PEPT1.[5] Nevertheless, modulation of intestinal absorption of lisinopril may influence its pharmacokinetics.

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Green tea catechins, especially (–)-epigallocatechin gallate (EGCG), have received significant attention for its beneficial effects on health, including antiviral effects.[6] A recent meta-analysis revealed that green tea consumption is associated with a reduced risk of cardiovascular outcomes and total mortality.[7] Thus, it is quite likely that patients undergoing antihypertensive or heart failure therapy habitually consume green tea or catechin supplements. However, at present, it remains largely unknown whether green tea consumption affects the pharmacokinetics as well as therapeutic outcomes of cardiovascular drugs. EGCG and other catechins have been reported to inhibit drug metabolism enzymes and transporters, such as cytochrome P450 (CYP), organic anion transporting polypeptides (OATPs), and P-glycoprotein.[8–12] Moreover, previous studies by our group have demonstrated that green tea and EGCG-concentrated green tea extract (GTE) significantly reduced plasma concentrations of a hydrophilic β-blocker, nadolol, in humans, probably by inhibiting the intestinal absorption of nadolol.[12–14] These findings pose a hypothesis that the pharmacokinetics of hydrophilic cardiovascular drugs could be susceptible to green tea consumption.

Of particular interest, lisinopril has similar pharmacokinetic property to nadolol, such as oral bioavailability (ca. 30%), relatively longer-half life, and resistance to metabolism. In addition, taking into account a much higher number of prescriptions of lisinopril, it is reasonable to assume that more patients treated with lisinopril consume green tea or catechin supplements aiming to reduce the risk for cardiovascular events. Therefore, in the present study, we investigated whether a single co-administration of lisinopril with an aqueous solution of EGCG-concentrated GTE influences the pharmacokinetics of lisinopril in healthy volunteers.

METHODS

Study design

An open-label, randomized, single-center crossover study was carried out in two phases, separated by a washout period of > 1 week. Twelve healthy volunteers (7 men and 5 women; aged 21–29 years; body mass index 18.6–23.1 kg/m^2) participated in the study after giving written informed consent. The volunteers were ascertained to be healthy by medical history, physical examination, and routine laboratory tests before entering the study. None of the volunteers used continuous medication. Subjects were instructed to refrain from consuming green tea and fruit products, including apple, grapefruit, and orange juices for 7 days before lisinopril administration, and fasted the previous night.

As a commercially available decaffeinated GTE, Sunphenon-EGCG (Taiyo Kagaku, Yokkaichi, Japan) containing 92.5% (w/w) of EGCG, was used, and an aqueous GTE solution was made by dissolving 325 mg of GTE in 200 mL of water with stirring. A single oral dose of 10 mg lisinopril (Zestril; AstraZeneca, Osaka, Japan) was administered in the morning of the study day with 200 mL of an aqueous solution of GTE or with 200 mL of water (control). At 1 and 4 hours after the administration, a standardized snack and a lunch meal were provided, respectively. Blood samples (5 mL) were collected at 0, 0.5, 1, 2, 3, 4, 6, 8, and 24 hours after lisinopril administration and were immediately centrifuged at 2,000 g for 10 minutes at 4°C. Urine was collected during periods of 0–4, 4–8, and 8–24 hours after the administration. Plasma and urine samples were stored at –80°C until analysis. To assess the acute hemodynamic response to lisinopril, pulse rate (PR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were recorded in a sitting position after lisinopril administration using an automatic blood pressure monitor (HEM-7051-HP; Omron Healthcare, Kyoto, Japan). Based on a previous pharmacokinetic study, it was calculated that a sample size of 12 subjects would provide 80% power (α-level of 5%) to determine a 40% difference in the lisinopril area under the plasma concentration-time curve (AUC) between two phases.[2] The study protocol was reviewed and approved by the ethics committee of Fukushima Medical University, and was registered at the UMIN Clinical Trials Registry as UMIN000030894. The study procedures were in accordance with the ethical standards of the Declaration of Helsinki.

Determination of lisinopril concentration

The concentrations of lisinopril in plasma and urine were determined by high-performance chromatography with tandem mass spectrometry (AB Sciex, Framingham, MA) as described in detail in **Supplementary Information**. The limit of quantification of lisinopril was 1 ng/mL, and the calibration curve ranged from 1–500 ng/mL. The interassay accuracy was within 100 ± 8.6%, with coefficient of variation of < 7.4%.

Data and statistical analysis

Pharmacokinetic data are expressed as geometric means and 90% confidence interval (CI). Pharmacokinetic analysis was performed by noncompartmental model using WinNonlin (version 5.1; Certara, Princeton, NJ), and maximum concentration (C_{max}) and time to C_{max} (T_{max}) of lisinopril were obtained by inspection. The AUC was estimated for the observed values by the trapezoidal method. The amount of lisinopril excreted into urine for 24 hours (A_e) was calculated as lisinopril urinary concentration multiplied by urine volume in each time duration. The renal clearance (CL_{renal}) was obtained from the equation $CL_{renal} = A_e/AUC_{0–24}$. Log-transformed pharmacokinetic parameters excluding T_{max} were compared between the control and GTE phases by paired t-test. Wilcoxon signed-rank test, using GraphPad Prism (version 6.07; GraphPad Software, San Diego, CA), was performed for T_{max}. A P value of < 0.05 was considered statistically significant.

RESULTS

Ten subjects completed the study with no adverse events, and two male participants withdrew from the study because of personal reasons.

Plasma concentration-time profiles and pharmacokinetic parameters of lisinopril are shown in **Figure 1a** and **Table 1**, respectively. Lisinopril C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ in the GTE phase were significantly decreased by 71% ($P < 0.001$), 69% ($P < 0.001$), and 67% ($P < 0.001$), respectively, compared
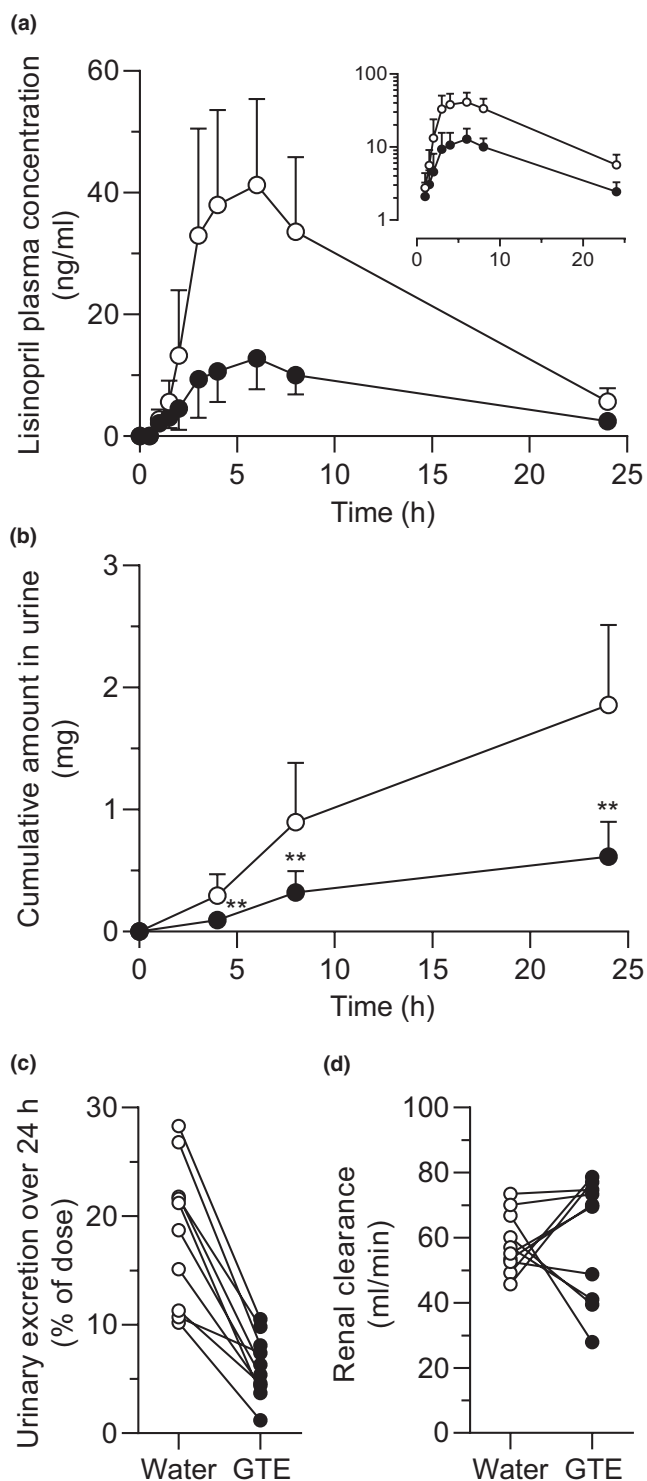
(a)

(b)

(c)

(d)

Figure 1 Effect of green tea catechin ingestion on the pharmacokinetics of lisinopril. Plasma concentrations (**a**), mean cumulative amount excreted into urine (**b**), % of dose over 24 hours (**c**), and renal clearance (**d**) of lisinopril after an oral administration (10 mg) with 200 mL of an aqueous solution of green tea extract (GTE) (●), or water (control, ○) in 10 healthy volunteers. Green tea extract contained approximately 300 mg of (–)-epigallocatechin gallate. Insert displays the same data on a semilog plot. Data are expressed as mean ± SD. **$P < 0.01$ with respect to control phase.

with values in the control phase. The geometric mean ratio (GTE/control) for C_{max} and $AUC_{0-\infty}$ of lisinopril were 0.289 (90% CI 0.226–0.352) and 0.337 (90% CI 0.269–0.405), respectively. Individually, the decreases in lisinopril AUC in the GTE phase were highly correlated with lisinopril AUC in the control phase ($r = -0.954$, $P < 0.001$). The amount of lisinopril excreted into urine over 24 hours in the GTE phase was significantly reduced by 69% ($P < 0.001$) as compared with the control phase (**Figure 1b,c**). However, no changes were observed in T_{max} or CL_{renal} between the two phases (**Figure 1d** and **Table 1**).

The baseline values of PR, SBP, and DBP in subjects were 73 ± 8 beats/minute, 107 ± 13 mmHg, and 68 ± 10 mmHg (mean ± SD), respectively. As for pharmacodynamic responses to lisinopril, DBP was lowered in both phases with mean maximum decrease of about 20% from baseline (52 ± 5 mmHg in control and 55 ± 4 mmHg in GTE) at 6 hours after the administration (**Figure S1**). Lisinopril tended to decrease SBP but increase PR by a single oral dose, however, there were no differences in SBP and PR between the control and GTE phases.

DISCUSSION

This study demonstrates for the first time that oral administration of lisinopril with an aqueous solution of EGCG-concentrated GTE resulted in a marked decrease in plasma concentrations and urinary excretion of lisinopril when compared with control (water) in healthy volunteers. Consistent with previous clinical pharmacokinetic studies of lisinopril,[2,15,16] there was significant intersubject variation in pharmacokinetic parameters with relatively higher coefficient of variation values (**Table 1**). Subjects who showed a greater reduction in the lisinopril AUC in the GTE phase had a higher AUC value in the control phase, indicating that individuals who originally have better exposure to lisinopril are more profoundly affected by GTE ingestion. Taking into account that there was no difference in CL_{renal} between the GTE and control phases, it is suggested that EGCG mainly inhibits the intestinal absorption of lisinopril, as is the case for nadolol–GTE interaction.[13]

To date, little is known about clinical drug-drug interactions (DDIs) of lisinopril. Rather, lisinopril may be regarded as a low-DDI risk drug because (i) there is no possibility of CYP-mediated interactions with lisinopril, and (ii) previously reported DDIs of lisinopril with food or drugs, such as digoxin and nifedipine, were negligible.[15–17] However, due to high solubility, low membrane permeability, and poor metabolism, lisinopril is classified as a class 3 drug in both Biopharmaceutics Classification System and Biopharmaceutics Drug Disposition Classification System, which suggested that absorptive transporter effects may dominate the pharmacokinetics.[18] We designed this study based on our previous findings on nadolol–green tea interaction with respect to the following points: (i) EGCG would be a contributing factor to the interaction among various components of green tea, and (ii) single concomitant ingestion of GTE or green tea containing 300 mg of EGCG might be enough to cause the interaction.[12–14] The concentration of total catechins in a typical brewed green tea beverage has been reported to be 110–210 mg/100 mL,[19] and therefore a cup (200 mL) of green tea contains about 220–420 mg of

Table 1 Pharmacokinetic parameters of lisinopril after oral administration with an aqueous solution of GTE or water (control) in healthy volunteers

	Control		GTE	
	Geometric mean	CV	Geometric mean	CV
C_{max}, ng/mL	42.2	37.2	12.2	36.6
GMR (90% CI)			0.289	(0.226–0.352)
T_{max}, hour	6.0	(3.0–6.0)	6.0	(3.0–8.0)
AUC_{0-24}, hour ng/mL	504.1	34.0	156.7	32.8
GMR (90% CI)			0.311	(0.247–0.375)
$AUC_{0-\infty}$, hour ng/mL	553.6	34.0	186.5	30.3
GMR (90% CI)			0.337	(0.269–0.405)
A_e, mg	1.75	35.3	0.54	46.7
GMR (90% CI)			0.307	(0.224–0.389)
CL_{renal}, mL/min	57.7	15.7	56.9	31.2
GMR (90% CI)			0.986	(0.774–1.198)

Lisinopril (10 mg) was orally administered with 200 mL of an aqueous solution of GTE, or with water (control) in 10 healthy volunteers. The T_{max} is expressed as median (range).
A_e, amount excreted unchanged into urine over 24 hours; AUC, area under the plasma concentration-time curve; AUC_{0-24}, AUC from zero to 24 hours; $AUC_{0-\infty}$, AUC from zero to infinity; CI, confidence interval; C_{max}, peak plasma concentration; CL_{renal}, renal clearance; CV, coefficient of variation; GMR, geometric mean ratio; GTE, green tea extract; T_{max}, time to C_{max}.

catechins. Assuming that EGCG dose-dependently affects the lisinopril pharmacokinetics, as we observed in nadolol–EGCG interactions,[13] it is likely that EGCG at smaller doses contained in green tea products and supplements could substantially decrease the plasma concentrations of lisinopril in real-world settings.

It is of interest to clarify the detailed mechanisms underlying lisinopril-EGCG interaction. To our knowledge, there is no direct evidence that lisinopril is a substrate of intestinal uptake transporters. PEPT1 can be one candidate, however, unlike lipophilic ACE inhibitors, such as fosinopril and zofenopril, lisinopril exhibits very weak affinity to PEPT1, suggesting that lisinopril could be a poor or nonsubstrate of PEPT1.[5,20] In addition, there is no report of EGCG inhibiting PEPT1 activity, whereas (–)-epicatechin gallate, another gallate catechin, not inhibiting the uptake transport of glycyl-sarcosine, a typical substrate of PEPT1, in Caco-2 cells.[21] This suggests that catechins may display only a weak inhibitory potential on PEPT activity. OATPs could also be candidate influx transporters, because EGCG and (–)-epicatechin gallate have been shown to inhibit OATP1A2, OATP1B1, and OATP2B1 activities *in vitro*.[11,12] For instance, OATP1B1 can transport enalapril and temocapril, and may contribute to the hepatic clearance of these ACE inhibitors in humans.[22,23] Accordingly, it is worthwhile to identify possible drug transporter(s) responsible for lisinopril membrane trafficking in enterocytes, including OATP1A2 and OATP2B1, which have been reported to be expressed in human intestine.[24–26]

Among ACE inhibitors, lisinopril has a unique property that does not require hydrolysis to exert ACE inhibition.[27] In other words, ACE inhibitors except for lisinopril and captopril are ester prodrugs, and thus they are more lipophilic in order to increase the intestinal absorption, and are enzymatically hydrolyzed in the liver to active metabolites by carboxylesterase 1. Hence, it is unclear whether green tea or catechins could affect the pharmacokinetics of ACE inhibitors other than lisinopril. Additionally, it remains to be elucidated whether other dietary flavonoids, such as

naringin and/or fruit juices, also modulate the intestinal absorption of lisinopril. Another aspect that may limit the interpretation of clinical results is that the participants of this study were all young Japanese adults, and thus it cannot rule out the possibility of ethnic differences in the pharmacokinetics of lisinopril. Last, this study only observed acute pharmacokinetic changes and hemodynamic response to lisinopril after a single co-administration with GTE. Therefore, larger studies are needed to evaluate whether the effect of green tea consumption on the lisinopril pharmacokinetics could influence long-term therapeutic outcome in patients.

In conclusion, the present study demonstrated that a concomitant ingestion of EGCG-concentrated GTE significantly decreased oral bioavailability of lisinopril without altering renal clearance of lisinopril. Patients should avoid consumption of green tea or its catechin products during lisinopril treatment. Particularly in countries where green tea is routinely consumed by patients, the impact of green tea on the lisinopril pharmacokinetics as well as its therapeutic efficiency could not be ignored.

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