Pharmacokinetics of Gallic Acid and Its Relative Bioavailability from Tea in Healthy Humans[1]

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ABSTRACT Gallic acid (GA), a food component that is especially abundant in tea, is an antimutagenic, anticarcinogenic and anti-inflammatory agent. We conducted a study using acidum gallicum tablets that contained 10% GA and 90% glucose and a black tea brew that contained 93% of its GA in free form to determine the pharmacokinetics and relative bioavailability of GA in healthy humans. After the administration of a single oral dose of acidum gallicum tablets or tea (each containing 0.3 mmol GA) to 10 volunteers, plasma and urine samples were collected over various time intervals. Concentrations of GA and its metabolite, 4-*O*-methylgallic acid (4OMGA), were determined, and the pharmacokinetic parameters were calculated. GA from both the tablets and tea was rapidly absorbed and eliminated with mean half-lives of 1.19 ± 0.07 and 1.06 ± 0.06 h and mean maximum concentrations of 1.83 ± 0.16 and 2.09 ± 0.22 μmol/L (plasma), respectively. After oral administration of the tablets and black tea, 36.4 ± 4.5 and 39.6 ± 5.1% of the GA dose were extracted in urine as GA and 4OMGA, respectively. The relative bioavailability of GA from tea compared with that from the tablets was 1.06 ± 0.26, showing that GA is as available from drinking tea as it is from swallowing tablets of GA. J. Nutr. 131: 1207–1210, 2001.

KEY WORDS: • tea • gallic acid • bioavailability • humans • HPLC

Gallic acid (GA)[3] is an endogenous product found in plants (1–3), and in free or bound forms, it is found in large amounts in tea leaves (3), from which it is extracted in hot water infusions.

GA is a strong antioxidant that possesses antimutagenic and anticarcinogenic activities (4–10). Its derivative, 4-*O*-methylgallic acid (4OMGA), has been reported as the main metabolite of GA in rats, rabbits and chickens (1,11–15) and humans (16), but despite the special activities of GA, there are no data available about the extent of its absorption, elimination or relative bioavailability from foodstuffs like tea, which is one of the most common drinks for humans. To estimate such data, our study was performed in healthy adult humans.

As a source of pure GA, acidum gallicum tablets were used that contained 10% GA and 90% glucose. Acidum gallicum tablets have been in use for more than four decades as therapy for patients with bronchitis (17). Here, we compare the bioavailability and some pharmacokinetic parameters of GA from a single oral dose of acidum gallicum tablets with that of tea.

SUBJECTS AND METHODS

Subjects. Each trial was performed with 10 healthy volunteers (5 men and 5 women) with ages ranging from 23 to 36 y (mean: 28 y), with body weights of 47–82 kg (mean: 63 kg) and heights of 152–190 cm (mean: 171 cm). They did not use any medication. The study was approved by the Ethics Commission of Justus Liebig University Giessen, and the subjects were fully informed of the protocol and intent of the study. The acute toxicity of GA is very low; 24–29 mmol/kg subcutaneous or intraperitoneal is required for a 50% lethal dose to mice or rats. Prolonged consumption in amounts well above normal food levels is not known to produce untoward effects (1).

Study design. For a bioavailability study involving tea GA, we determined free and bound GA in different kinds of tea. To avoid the possible interference of conjugated forms of GA such as theogallin (5-*O*-galloyl-quinic acid), (−)-epigallocatechin-3-gallate or (−)-epicatechin-gallate in this study, we chose and used Assam black tea extract, which contains ~93% of its GA in free form.

Tea or acidum gallicum was administered at 0800 h after an overnight fasting. On the day before and during the study, subjects were asked to consume a GA-free diet, which consisted of white bread, cheese and water. In the first trial, 2 acidum gallicum tablets [0.15 mmol (25 mg) GA in a single tablet] were swallowed with 200 mL water. In the second trial, the tea brews were prepared immediately before administration. Each time, 125 mL Assam black tea brew containing 0.3 mmol (50 mg) free GA was adjusted with distilled water to 200 mL and ingested orally.

Blood samples (10 mL) were collected into heparinized blood containers (KABE Labortechnik, Nuembrecht-Elsenroth, Germany) before the administration and at different time intervals (i.e., 0.67, 1.33, 2, 3, 4, 5.5, 7.75 and 12 h) after the dose. The samples were centrifuged, and the harvested plasma was frozen immediately and stored at −18°C until analyzed. Urine was collected before dosing and quantitatively during the 24 h after dosing. Total urine volume was measured for each collection period, and 1-mL aliquots were stored frozen until assayed for GA and 4OMGA.

Plasma and urine samples were analyzed for GA and its metabolite, 4OMGA, using a previously reported HPLC method (16). The preparation procedure for the samples was the same as described for analysis of the tea brews. The plasma samples were centrifuged at 1800 × *g* for 10 min after extraction with ethyl acetate and before separation of the organic fraction. The limit of quantification was 0.30 μmol/L, and a limit of detection (signal-to-noise ratio of 3) of 0.15 μmol/L was obtained in both predose human plasma and human urine samples.

Tea brews. Commercial samples (25 g) of Darjeeling, Assam (CTC Numalighur), Sri Lanka and Chinese green tea were put into a pot, extracted with 500 mL hot water and brewed on a hot plate for

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[3] Abbreviations used: AUC, area under the plasma concentration-time curve; GA, gallic acid; 4OMGA, 4-*O*-methylgallic acid.

TABLE 1

Mean gallic acid (GA) and 4-O-methylgallic acid (4OMGA) pharmacokinetic parameters after the administration of a single 0.3 mmol (50 mg) oral dose of GA as two acidum gallicum tablets or tea to 10 healthy adults[1]

	Acidum gallicum tablet		Assam black tea	
	GA	4OMGA	GA	4OMGA
C_{max},[2] *μmol/L*	1.83 ± 0.16	2.83 ± 0.25	2.09 ± 0.22	2.64 ± 0.28
t_{max}, *h*	1.27 ± 0.20	1.51 ± 0.31	1.39 ± 0.21	1.46 ± 0.27
$AUC_{(0–12h)}$, $\mu mol \cdot h \cdot L^{-1}$	4.29 ± 0.81	9.56 ± 1.84	4.55 ± 0.80	9.02 ± 1.52
$t_{1/2}$, *h*	1.19 ± 0.07	1.50 ± 0.09	1.06 ± 0.06	1.31 ± 0.06
k, h^{-1}	0.58 ± 0.03	0.46 ± 0.03	0.65 ± 0.04	0.53 ± 0.02
A_e, *μmol*	35.9 ± 7.6	65.7 ± 10.3	38.2 ± 6.5	71.2 ± 12.5
CL_r, *L/h*	8.4 ± 2.4	6.9 ± 1.7	8.4 ± 2.0	7.9 ± 1.9

[1] Values are means ± SD, $P < 0.005$.

[2] C_{max}, maximum plasma concentration; t_{max}, maximum time; AUC, area under the plasma concentration-time curve; $t_{1/2}$, half-life; *k*, elimination rate constant; A_e, total amount of GA or 4OMGA collected in urine; CL_r, renal clearance.

5 min (the procedure was conducted with a coffee machine). The brew was filtered, and 10 mL of every tea brew was used for determination of its free and bound GA and 4OMGA concentrations.

To extract GA and 4OMGA, 2 mL of 1 mol sulfuric acid/L was added to 2 mL tea brew in glass tubes. The tubes were put in a boiling water bath, and the samples were hydrolyzed for 30 min and then cooled in a cold-water bath. These samples and two nonhydrolyzed samples were subjected to extraction twice with a 4-fold volume of ethyl acetate. The organic phase was pipetted in round-bottom flasks, and the combined ethyl acetate extracts were evaporated to dryness under vacuum with a rotary evaporator, with the path temperature maintained at <35°C. Each extract was redissolved in an appropriate amount of HPLC mobile phase before HPLC analysis.

To validate the extraction method, 0.03, 0.3, 0.5, 3 and 6 μmol GA and 4OMGA were added to 10-mL tea brews. Then, 2 mL of each sample before hydrolysis and 2 mL after hydrolysis were extracted and analyzed. This procedure was repeated twice for each tea.

Chromatographic condition. The chromatographic condition (16) was the same for all samples (tea, plasma and urine samples). A Merck and Hitachi high-precision pump (model L-6000; Darmstadt, Germany) equipped with a 100-μL loop was used. The substances were detected using a Gynkotek spectrophotometer (SP-4; Germering, Germany) at 220 and 270 nm. Separation was carried out using a LiChrospher 100 RP-18 column (5 μm, 120 × 4 mm I.D.; Merck) with a guard column (RP-18, 4 × 4 mm; Merck). Chromatographic data were recorded using a Merck and Hitachi D-2000 chromatointegrator. The chromatographic separation of GA and 4OMGA was achieved by reverse phase HPLC using isocratic elution. Mobile phase was water/acetonitrile (97.5:2.5, v/v) modified with 2.5 mol phosphoric acid/L. Retention times and absorbance ratios (at two different wavelengths: 220 and 270 nm) against those of standards were used to identify the separated peaks and to check their purity. Quantitative determinations were carried out with the external standard method.

Data analysis and statistics. Noncompartmental pharmacokinetic parameters were calculated from plasma concentration-time data using established methods (with computer software package TopFit 2) (18,19). The maximum plasma concentration was determined through visual inspection of the data. Area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule. Beyond 12 h, the area was in all cases insignificant. If the concentration after 12 h (C_{12}) was below the quantification limit, C_{12} was determined with the equation $C_{12} = C_{last} \times e^{-kt}$, where *t* is $12 - t_{last}$, and C_{last} and t_{last} are the last measurable concentration and the respective time. The elimination rate constant (*k*) and half-life were calculated from log-linear regression of the plasma concentration-time profile in the terminal portion of the curve. Total urinary recovery was determined by multiplying the concentration of parent GA or its metabolite (4OMGA) in urine by the volume of the urine sample in each collection interval and then calculating the sum for all intervals after dosing. Renal clearance was calculated by dividing the total amount of parent GA or 4OMGA collected in urine by the respective plasma AUC during the 12-h interval. Relative bioavailability was assessed by the tea-to-acidum gallicum ratios of the plasma $AUC_{(0–12\ h)}$.

Results are means ± SD. The statistical significance of differences was tested using the nonparametric Wilcoxon test, and testing for sequence effects was performed with ANOVA with the Bonferroni correction. The differences were considered statistically significant when the calculated *P*-value was <0.05.

RESULTS AND DISCUSSION

Determination of GA and 4OMGA in tea samples. A study of the repeatability of the analytical method and its reproducibility between days was performed. The repeatability ($n = 7$) showed a relative standard deviation of 2%. The reproducibility ($n = 10$) between days was 3.5%, and good response linearity was obtained between 0.1 and 300 μmol/L (in water). Recoveries of both GA and 4OMGA from tea samples exceeded 93%. The results of comparisons of different tea samples showed that Assam black tea brew contained the lowest amount of conjugated GA; 93% of its GA was in free form. (The brew of 25 g tea leaves extracted with 500 mL hot water contained 2.35 ± 0.08 and 0.18 ± 0.03 mmol free and conjugated GA/L, respectively; $n = 4$.) Therefore, it was used for pharmacokinetic and bioavailability studies. In the Chinese green tea, 97% of GA was in its conjugated forms. We did not find any 4OMGA in the tea samples.

Pharmacokinetics and bioavailability. The results of the noncompartmental pharmacokinetic analysis are listed in **Table 1**. **Figure 1** shows the chromatograms of plasma before and after the administration of acidum gallicum and Assam black tea brew. The mean plasma concentration-time profiles of GA and 4OMGA after the administration of acidum gallicum and tea are shown in **Figure 2**.

The oral absorption of GA from both sources was fast (t_{max}: 1.27 ± 0.20 h for acidum gallicum tablets and 1.39 ± 0.21 h for the tea). The sum of GA excreted in urine as unchanged GA and its metabolite, 4OMGA, was 36.4 ± 4.5% for acidum gallicum tablets and 39.6 ± 5.1% for the tea ($P > 0.05$). There was no difference in the $AUC_{0–12}$ calculated for tea and acidum gallicum. With respect to mean AUC, the calculated relative bioavailability was 1.06 ± 0.26, which shows that GA is as available from drinking tea as it is from swallowing tablets of GA.

The effects of tea consumption on health have recently received a great deal of attention. Many laboratory studies

have demonstrated clearly and repeatedly the inhibition of tumorigenesis in different animal models by tea and tea polyphenols (10,20–24).

The molecular mechanisms for these inhibitory actions are not fully understood. A major problem in investigating the relationship between tea and cancer is the lack of quantitative data. Even in studies with animals, mechanistic understanding of the inhibitory effect of tea against tumorigenesis is hampered by a lack of information on the bioavailability of the effective components of tea. It has been assumed that most of the cancer-inhibitory activity of tea is due to the polyphenols present in the tea. GA has been suggested as a biomarker for tea consumption. It was found that the GA moiety of theaflavins is essential for their potent antioxidative and antimutagenic activities (24).

In this study, we obtained some detailed information about the bioavailability of GA of tea in humans. We prepared 200 mL tea brew of 6.24 g black tea leaves (Assam), which was about three times more concentrated than normal tea brews. GA concentration in the stomach could achieve a maximum of 1.5 mmol/L (there was 0.3 mmol GA in 200 mL tea brew). GA was rapidly absorbed, but the highest GA concentration observed in plasma was only 1.83 ± 0.16 μmol/L for the tablets and 2.09 ± 0.22 μmol/L for the tea after 1.3 ± 0.2 and 1.4 ± 0.2 h, respectively. In addition, the highest concentration of its metabolite in plasma was not higher than 2.83 ± 0.25 μmol/L for the tablets and 2.64 ± 0.28 μmol/L for the tea (t_{max} = 1.5 ± 0.3 h). Whether these low plasma concentrations of GA can have pharmacological activity in the body needs to be investigated. Although with respect to the total amount collected in the urine, >60% of GA excreted was metabolized to 4OMGA, there is no information about whether this metabolite can contribute to the pharmacological effects. Therefore, a greater understanding of pharmacological activities of 4OMGA is highly desirable.

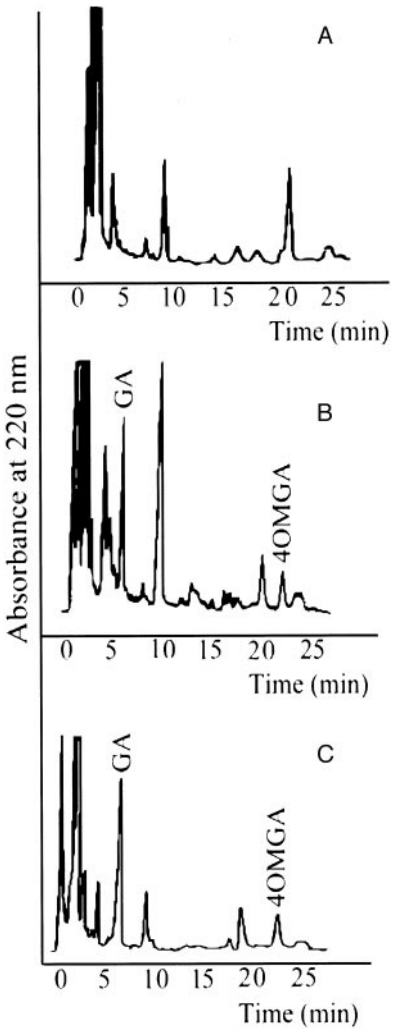

FIGURE 1 Chromatograms obtained by HPLC in human plasma (*A*) before and (*B*) 90 min after the administration of 2 acidum gallicum tablets containing 0.3 mmol gallic acid (GA) and 90 min after the administration of Assam black tea brew containing 0.3 mmol GA (*C*). 4OMGA, 4-*O*-methylgallic acid.

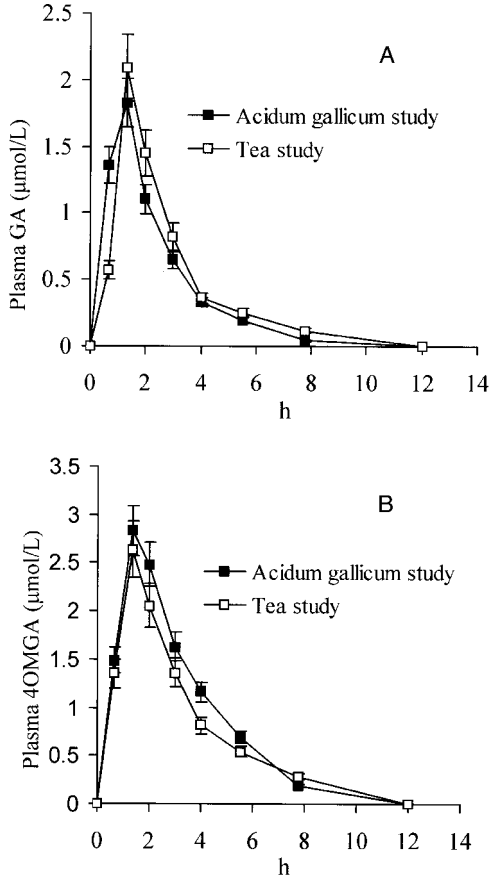

FIGURE 2 Plasma concentration-time profiles of (*A*) gallic acid (GA) and (*B*) 4-*O*-methylgallic acid (4OMGA) after the administration to humans of a single 0.3-mmol oral dose of GA from 2 acidum gallicum tablets and from Assam black tea. Values are mean ± SE, n = 10.

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