JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Intestinal Absorption of *p*-Coumaric and Gallic Acids in Rats after Oral Administration

YUTAKA KONISHI,*,† YOSHITAKA HITOMI,† AND EIJI YOSHIOKA‡

Applied Bioresearch Center, Research & Development Department, Kirin Brewery Co., Ltd., 3 Miyaharacho, Takasaki-shi, Gunma 370-1295, Japan, and Pharmaceutical Development Laboratories, Pharmaceutical Division, Kirin Brewery Co., Ltd., 2-2 Souja-machi 1 chome, Maebashi-shi, Gunma 371-0853, Japan

Ferulic acid (FA) and *p*-coumaric acid (CA) are absorbed by the monocarboxylic acid transporter (MCT) in Caco-2 cells, although gallic acid (GA) is not. Therefore, the MCT is selective for certain phenolic acids. Absorption of orally administered CA and GA in rats was studied to obtain serum pharmacokinetic profiles and to investigate their intestinal absorption characteristics in vivo. Rats were administered 100 μmol/kg body weight of CA and GA, and blood was collected from the portal vein and abdominal artery after administration. CA, GA, and their metabolites were quantified with a highly selective and sensitive coulometric detection method using high-performance liquid chromatography−electrochemical detection. Ingested CA was rapidly absorbed in the gastrointestinal tract in an intact form. The serum concentration of intact CA in the portal vein peaked 10 min after dosing (C_{max} was 165.7 μmol/L). In contrast, GA was slowly absorbed, with a t_{max} for intact GA of 60 min and a C_{max} of 0.71 μmol/L. The area under the curve for intact CA and GA was calculated from the serum concentration profile in the portal vein to be 2991.3 and 42.6 μmol min L^{-1}, respectively. The relative bioavailability of CA against GA was about 70. This is the first demonstration that absorption efficiency of CA is much higher than that of GA in vivo. The absorption characteristics of CA are clearly different from those of GA. These findings are in good agreement with the results obtained in vitro using a Caco-2 cell system.

KEYWORDS: ***p*-Coumaric acid; gallic acid; monocarboxylic acid transporter; intestinal absorption; rats**

INTRODUCTION

Polyphenols are widely distributed in edible plants and are classified into phenolic acids, flavonoids, and the less common stilbenes and lignans. The bioavailability of these compounds depends on the rate and extent of intestinal absorption and the characteristics of the metabolites in the plasma (*1*). The total polyphenol intake has been reported to be in the order of 1 g/day (*1*). However, considerable uncertainty remains due to the lack of comprehensive data concerning some of the major polyphenol classes in food. Dietary polyphenols are widely considered to contribute to health benefits in humans; however, little is known concerning their bioactive forms in vivo and the mechanisms by which they may prevent disease. Many studies have focused on the absorption and metabolism of flavonoids. The absorptions of catechins (*2*−*4*), anthocyanins (*5*, *6*), flavones (*7*−*9*), flavonols (*8*), and their glycosylated forms have been examined. Ingested flavonoids were present in rat or human systemic blood circulation either in an intact form or as various metabolites. In contrast, few studies have investigated phenolic acids such as FA and caffeic acid (*10*−*12*), despite their high content in fruits, cereals, and some vegetables (*13*).

The partition coefficient (log octanol/water) is thought to govern the intestinal absorption of dietary polyphenols, because only passive diffusion seems to be involved (*1*). We have reported that both FA and CA are transported across human intestinal Caco-2 cells by the MCT (*14*, *15*), as is the case for fluorescein (*16*). In contrast, GA is not transported by MCT but permeated via a paracellular route in Caco-2 cells; the permeation rate is about 100 times lower than that of CA (*15*). Phenolic acids such as CA and GA (**Figure 1**) are antioxidants (*17*) exhibiting antimutagenic and anticarcinogenic activities (*18*−*24*). Until recently, only a few studies have described the absorption and metabolism of these two compounds (*25*, *26*). This study was designed to investigate the intestinal absorption characteristics of CA and GA in vivo and to compare their bioavailabilities with some pharmacokinetic profiles.

MATERIALS AND METHODS

Materials. CA, GA, benzoic acid (BA), mannitol, and sulfatase type H-5 were purchased from Sigma-Aldrich, Inc. (St. Louis, MO), and other chemicals used in this study were of analytical grade.

Animals and Diets. Male Sprague−Dawley rats (6 weeks old, SLC, Hamamatsu, Japan) weighing 170−205 g were housed in an air-

* To whom correspondence should be addressed. Tel: +81-27-346-9441. Fax: +81-27-346-9985. E-mail: konishiy@kirin.co.jp.
† Applied Bioresearch Center, Kirin Brewery Co., Ltd.
‡ Pharmaceutical Division, Kirin Brewery Co., Ltd.

10.1021/jf035366k CCC: $27.50
Published on Web 04/06/2004

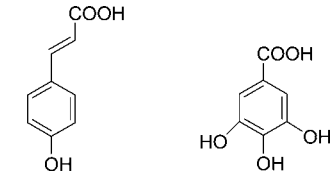

Figure 1. Chemical structures of CA and GA.

conditioned room (22 ± 4 °C) under 12 h dark/12 h light cycles, with free access to tap water and CE-2 diet (CLEA Japan, Inc., Tokyo, Japan). Three rats were assigned to each experimental group.

Sample Preparation. Rats were fasted for 20 h and then were administered CA or GA (100 μmol/kg in 10% propyleneglycol) by gastric intubation. Blood was withdrawn from the portal vein and abdominal artery at each time point after dosing. Serum was obtained by centrifugation and was stored at −80 °C until use.

High-Performance Liquid Chromatography (HPLC)-ECD Analysis. HPLC-ECD fitted with an coulometric detection system was performed according to the method described previously (*14*). To 25 μL of serum were added 25 μL of 0.1 mol/L sodium acetate buffer (pH 5.0) and 450 μL of 0.83 mol/L acetic acid in methanol. The mixture was vortexed for 30 s, sonicated for 30 s, and finally centrifuged at 8500*g* for 5 min at 4 °C. The supernatant was diluted with solvent A, and 25 μL was injected onto an HPLC C18 column (ODS150, MC Medical, Inc., Tokyo, Japan). The mobile phase A (solvent A) was 50 mmol/L sodium acetate containing 5% methanol (pH 3.0 adjusted with phosphoric acid), while mobile phase B (solvent B) was 50 mmol/L sodium acetate containing 40% acetonitrile and 20% methanol (pH 3.5 adjusted with phosphoric acid). To measure the amount of CA, the elution profile (0.6 mL/min) was as follows: 0−28.5 min, linear gradient from 85% solvent A/15% solvent B to 20% solvent A/80% solvent B; 28.5−31 min, isocratic elution 0% solvent A/100% solvent B; 31−35 min, isocratic elution 85% solvent A/15% solvent B. The eight electrode detector potentials were from 0 to 700 mV in increments of 100 mV. To measure the amount of GA, the elution profile (0.6 mL/min) was as follows: 0−5 min, isocratic elution 100% solvent A/0% solvent B; 5−28.5 min, linear gradient from 100% solvent A/0% solvent B to 20% solvent A/80% solvent B; 28.5−31 min, isocratic elution 0% solvent A/100% solvent B; 31−35 min, isocratic elution 100% solvent A/0% solvent B. The eight electrode detector potentials were from 0 to 700 mV in increments of 100 mV.

Enzymatic Hydrolysis and Determination of CA or GA Conjugates. Serum (25 μL) was mixed with 25 μL of sulfatase type H-5 solution in 0.1 mol/L acetate buffer (pH 5.0) containing both 12.5 units of sulfatase and β-glucuronidase activity (about 270 units). The mixture was incubated at 37 °C for 45 min. The difference of CA or GA content before and after sulfatase treatment was assumed to be the amount of respective sulfate and glucuronide conjugates in the sample (*4*).

Data Analysis. Noncompartmental pharmacokinetic parameters were calculated by the common method using serum concentration−time data with WinNonlin. The measured values were used for the maximum serum concentration, C_{max}. The area under the serum concentration−time curve ($AUC_{0-1.5h}$) in the portal vein from zero to the final sampling time (1.5 h) was calculated using the linear/log trapezoidal rule. The half-life ($t_{1/2}$) was calculated from log linear regression of the terminal phase of the serum concentration−time profile. Results are expressed as means ± standard errors of the mean (SEM). Statistical analysis was performed with one way analysis of variance followed by Fischer's test of least significant difference. Significance was set at $P < 0.05$.

RESULTS

Determination of CA and GA in Serum Samples. Figure 2 shows representative HPLC profiles of extract of serum from a control rat (**A**) and extracts of serum administered CA (**B**) and GA (**C**). On the basis of the comparison in two dimensions (i.e., chromatographic and voltammetric), the peak identity of CA or GA was determined by evaluating the peak area ratio for the adjacent oxidation channels (lower or upper) to the dominant oxidation channel. The voltammetric response of the analyte across these channels was unique for each compound, and more than 70% ratio accuracy was considered to support the peak purity (*27*). The retention time and dominant oxidation potential for CA and GA are 16.1 min and 600 mV and 7.4 min and 100 mV, respectively. Experiments with CA- or GA-spiked serum showed that this procedure gave >97% recovery in both cases. Quantitative determination of CA and GA was performed with an external standard method. It was verified that detector response was linear with standards up to 600 μmol/L for CA and 400 μmol/L for GA.

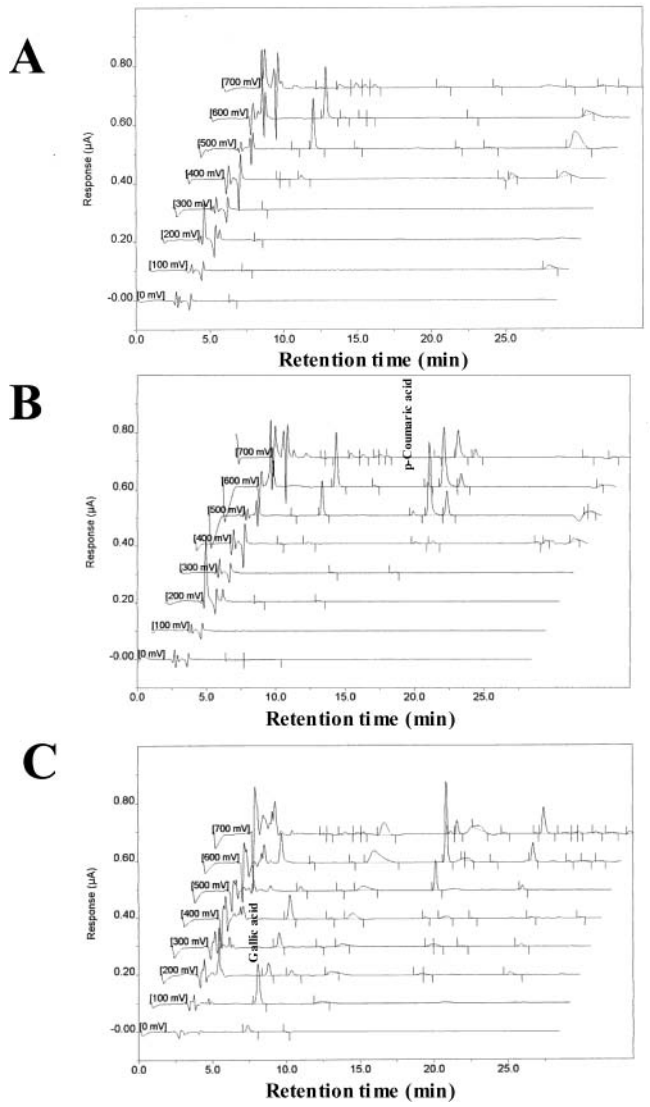

Figure 2. Chromatograms obtained by HPLC-ECD analysis in rat serum before (**A**) and after the administration of CA (**B**) and GA (**C**). The chromatogram from a control rat (**A**) was performed by the HPLC-ECD analysis according to the measurement for CA.

Quantitave Changes of CA, GA, and Their Metabolites in Rat Serum. The mean serum concentration−time profiles of CA, GA, and their metabolites in the portal vein after administration are shown in **Figure 3**. Total and intact concentrations of CA and GA were measured after and before deconjugation with sulfatase treatment, and the results of the noncompartmental pharmacokinetics analysis are listed in **Table 1**. The intestinal absorption of intact CA was extremely fast (t_{max}, 10 min; C_{max}, 165.7 ± 31.0 μmol/L), whereas intact GA was slowly absorbed (t_{max}, 60 min; C_{max}, 0.71 ± 0.28 μmol/L).

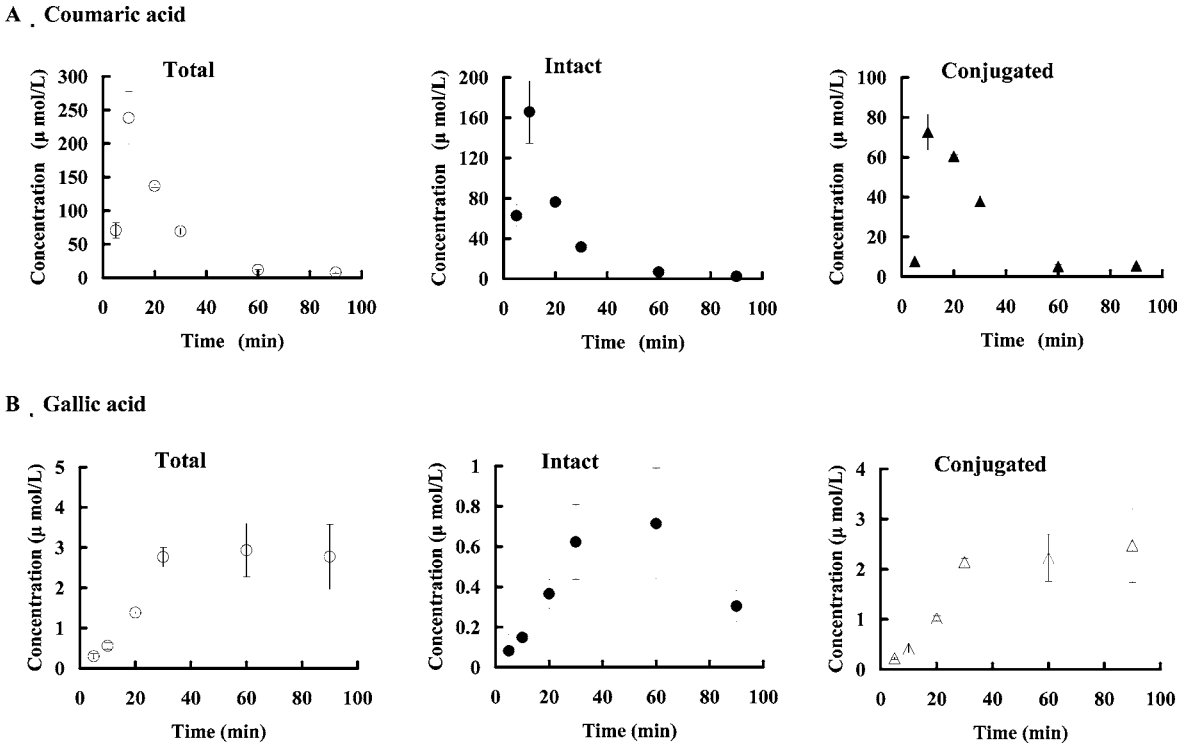

Figure 3. Serum concentration–time profiles of CA (**A**) and GA (**B**) in the portal vein after the administration. Values are means ± SE; $n = 3$.

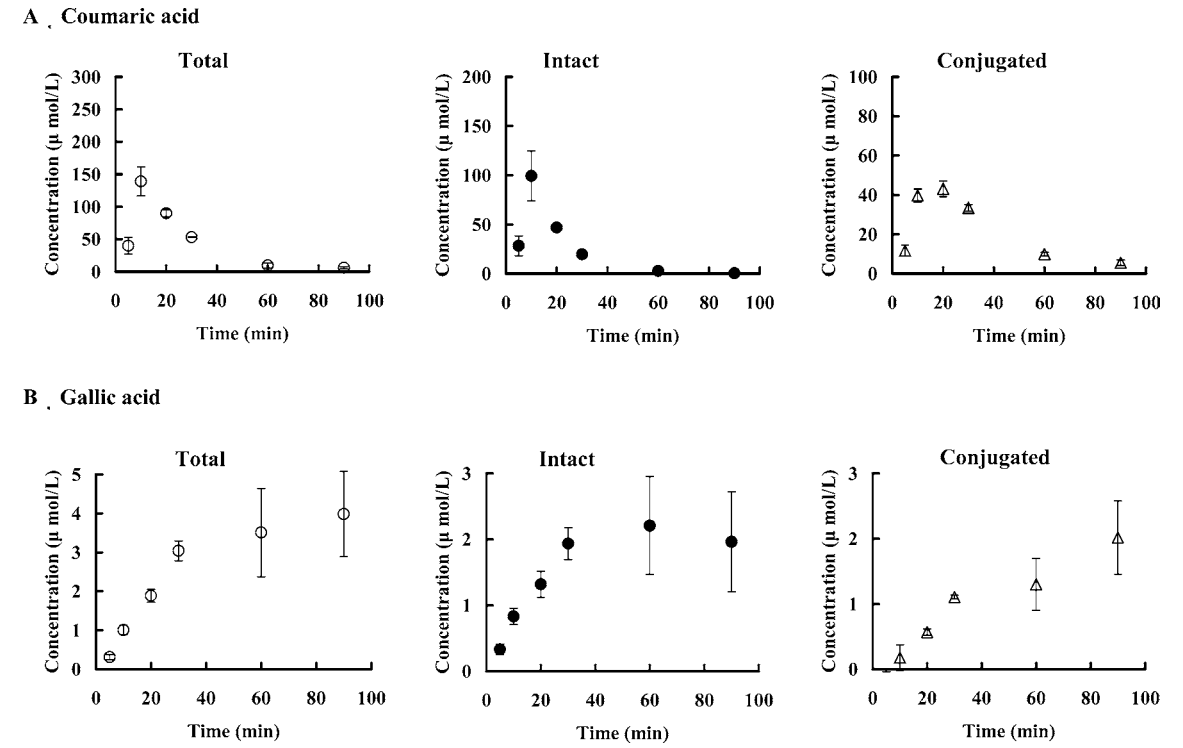

Figure 4. Serum concentration–time profiles of CA (**A**) and GA (**B**) in the abdominal artery after the administration. Values are means ± SE; $n = 3$.

In addition, intact CA was rapidly eliminated with $t_{1/2}$ of 15.9 min, although intact GA was eliminated more slowly than CA ($t_{1/2}$, 24.1 min). There was a significant difference in the AUC calculated for intact CA and GA. The relative bioavailability of CA against GA was estimated to be 70-fold greater. This result shows that the intestinal absorption efficiency of CA is much higher than that of GA (**Table 1**). Furthermore, the serum concentration profiles of CA, GA, and their metabolites in the abdominal artery were investigated to examine the hepatic elimination (**Figure 4**). The concentration of intact CA peaked at 10 min and decreased rapidly (t_{max}, 10 min; C_{max}, 99.3 ± 22.4 μmol/L), whereas intact GA changed gradually (t_{max}, 60 min; C_{max}, 2.21 ± 0.74 μmol/L). The concentrations of conjugated CA in the portal vein and the abdominal artery after 10 min were 72.6 ± 9.0 and 39.7 ± 3.3 μmol/L, whereas conjugated GA in the portal vein and the abdominal artery after 60 min showed 2.22 ± 0.47 and 1.30 ± 0.40 μmol/L (**Figures 3** and **4**). The concentration of CA was about 45-fold greater

Table 1. Pharmacokinetic Parameters of Intact CA and GA in the Portal Vein after the Administration of a Single 100 μmol/kg Oral Dose of CA and GA[a]

	CA	GA
C_{max} (μmol/L)	165.7	0.71
t_{max} (min)	10	60
$AUC_{0-1.5h}$ (μmol min L^{-1})	2991.3	42.6
$t_{1/2}$ (min)	15.9	24.1
relative bioavailability	70.2	

[a] Values are means. C_{max}, maximum serum concentration; t_{max}, maximum time; AUC, area under the serum concentration–time curve; $t_{1/2}$, half-life. Relative bioavailability was calculated as follows: AUC of CA/AUC of GA.

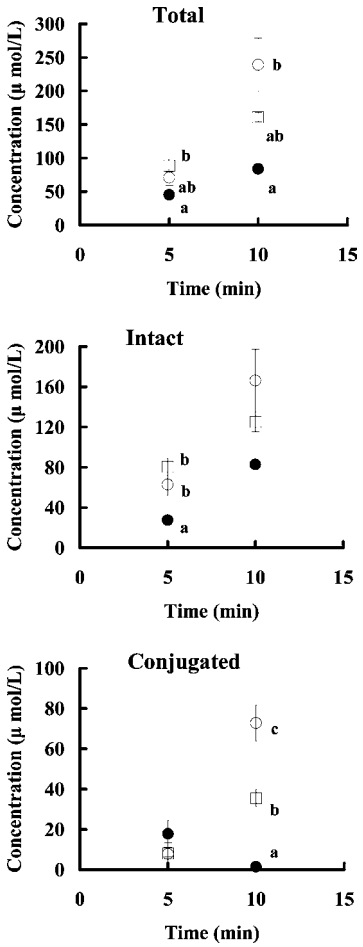

Figure 5. Serum concentration–time profiles of CA in the portal vein after the administration of CA alone (○), with BA (●), or with mannitol (□). Values are means ± SE; $n = 3$.

than that of GA in the abdominal artery indicating that much more CA was distributed to the whole body by the systemic circulation than GA, although a considerable amount of intact CA was eliminated in the liver.

We also investigated the effect of an excess amount of BA or mannitol on the absorption of CA. Rats were administered 100 μmol/kg body weight of CA with or without 10 mmol/kg body weight of BA or mannitol simultaneously (**Figure 5**). BA, a substrate of MCT (*28*, *29*), seemed to inhibit the absorption of intact CA. However, mannitol, a compound for paracellular diffusion, did not. Intriguingly, the amount of conjugated CA observed in the portal vein was decreased in dosing of both BA and mannitol; the effect of BA was greater than that of mannitol.

DISCUSSION

In a previous in vitro study using Caco-2 cells, we demonstrated that CA was transported by MCT. The absorption rate of CA was about 100 times greater than that of GA (*15*). The affinity of phenolic acids for MCT could depend on their structure. Hydroxylation of the substrate of MCT such as benzoic and cinnamic aicds would decrease the affinity for MCT because hydrogen bonding between the hydroxyl group of the substrate and MCT might interfere with the molecular recognition (*30*). Therefore, the great difference in absorption efficiency between CA and GA might be ascribed to obvious differences in their absorption characteristics (i.e., MCT-mediated absorption for CA and paracellular diffusion for GA) (*15*).

In this study, we have demonstrated that the absorption efficiency of CA was much higher than that of GA in vivo, indicating differences in the absorption characteristics of the two compounds (**Figure 3** and **Table 1**). These results are in good agreement with the conclusions of the in vitro study reported previously (*15*). It might be possible to ascribe the difference in absorption efficiency to the distinct transport characteristics in vivo, as was the case in the in vitro study (*15*). Solubility of ingested flavonoids was reported to affect the absorption and excretion significantly. Shimoi and coworkers reported that αG-rutin (a water soluble flavonoid) was absorbed more efficiently than either quercetin or rutin because the decrease of the precipitation in the alimentary tract raised the amount available for absorption (*8*). Solubility in the vehicle used for the oral administration also enhanced the absorption of flavonoids (*9*, *31*). On the contrary, CA was absorbed much more efficiently than GA in this study, although the solubility of GA was higher than that of CA considering the partition coefficients of CA or GA were 0.02 or 0.22, respectively (*17*). These may also suggest the differences in the absorption characteristics of CA and GA, and some specific mechanism would be involved in the absorption of CA in vivo. A considerable amount of conjugated CA and GA was observed in the portal vein (**Figure 3**), although no conjugated CA and GA was transported in Caco-2 cells (*15*). Spencer and coworkers, using the perfusion analysis of isolated intestine, reported that flavonoids and hydroxycinnamates were glucuronidated during permeation across rat epithelium without the need for gut (*25*). The discrepancy between the in vivo, in situ perfusion analysis and the in vitro Caco-2 cell system might originate from differences in the evaluation method itself.

Small doses of ingested polyphenols may be metabolized by intestinal mucosa, with the liver playing a secondary role to further modify large doses of the polyphenol conjugates from the small intestine in addition to excretion into bile. For example, in rats after an oral administration of 10 mg of (−)-epicatechin, the polyphenol was first glucuronidated during intestinal absorption, followed by hepatic sulfation and methylation (*4*). Comparing the serum concentration profiles in the abdominal artery with that in portal vein, the concentration profile of total and intact CA in the abdominal artery was similar to that in the portal vein, whereas the profile of conjugated CA seemed to be a little different from that in the portal vein (**Figure 3** and **Figure 4**). The conjugated CA peaked 20 min (t_{max}, 20 min; C_{max}, 43.1 ± 4.0 μmol/L). Intact CA, which evaded intestinal metabolism, might be metabolized in the liver. At 10 min after the administration, however, the ratio of intact CA in the portal vein to intact CA in the abdominal artery (165.7 vs 99.3 μmol/L) was quite similar to the ratio of conjugated CA (72.6 vs 39.7 μmol/L). These results seem to indicate that intact and conjugated CA were eliminated similarly in liver. By contrast,

the concentration profile of intact, conjugated, and total GA in the abdominal artery was almost the same as that in the portal vein (**Figure 3** and **Figure 4**), which suggested that GA was less susceptible to be eliminated in the liver than CA. There may be the difference in elimination characteristics in the liver, as was observed in the intestinal absorption. In contrast to GA, a large amount of intact CA possessing antioxidant activity is distributed to the whole body by systemic circulation. The greater absorption/distribution of ingested CA is likely to play a significant physiological role in preventing oxidative stress within the body. The biological properties of phenolic acids, such as CA, which are efficiently absorbed and distributed in the body, need to be studied in more detail.

The absorption of intact CA seemed to be inhibited by BA not but mannitol, whereas the conjugated CA was decreased in dosing of both BA and mannitol (**Figure 5**). These results, together with those obtained in the in vitro studies, suggest that some other absorption process (e.g., passive diffusion) might operate during the intestinal absorption of CA in vivo. BA might inhibit both the MCT-mediated and the passive diffusion transport of CA. However, the mechanism for the apparent decrease of conjugated CA transport by mannitol is unclear. Changes in the fluidity of the sample solution, caused by simultaneous dosing, might interfere with the access of CA to the cell. Perfusion studies to investigate simultaneous dosing might be necessary to reveal the absorption mechanism of CA.

Epigallocatechin gallate (EGCg), a major component of tea polyphenols, is widely assumed to contribute to health benefits; there is a wealth of information on its absorption, distribution, and excretion (*32*, *33*). The oral bioavailability of EGCg was found to be low (~2%) in animal and possibly in human (*32*, *33*). The AUC of EGCg (163 μmol/kg administered) was reported to be 38.0 μmol min L^{-1}, which was almost the same as that of GA observed in this study (**Table 1**). Furthermore, the permeability of EGCg (0.22 nmol/min/mg protein) across the intestinal Caco-2 cells seemed to be quite similar as that of GA (0.16 nmol/min/mg protein), both of which are permeated via the paracellular diffusion (*15*, *34*). Assuming that the absorption efficiency of GA was the same as that of EGCg, it might be possible that the percentage of CA and GA absorbed would be estimated to be ~100 and 1.6%, respectively, multiplying the percentage of EGCg absorbed (F, 1.6%) by relative bioavailability of CA against GA (*32*). Thus, we have speculated that the bioavailability of CA is much higher than that of GA also in human; however, further experiments are necessary to examine their bioavailability in humans considering the effect of food matrix ingested simultaneously.

In conclusion, we have demonstrated for the first time a higher efficiency of intestinal absorption of CA over GA in vivo. This finding supports the results obtained by in vitro studies using a Caco-2 cell system. The physiological effects of polyphenol on human health have recently received a great deal of attention. Many studies have been carried out to investigate the physiological role of flavonoid and isoflavonoids following ingestion. However, the bioavailability of these compounds is thought to be low (*1*). Because phenolic acids are ubiquitous in edible plants and are ingested on a daily basis through regular consumption of foodstuffs such as cereals and beer, a greater understanding of their pharmacological activity is highly desirable. Further studies on their physiological activity per se and on the effect of interactions with other polyphenolic compounds are necessary to clarify the benefits of dietary polyphenols on human health.

ABBREVIATIONS USED

MCT, monocarboxylic acid transporter; FA, ferulic acid; CA, *p*-coumaric acid; GA, gallic acid; BA, benzoic acid; ECD, electrochemical detector.

ACKNOWLEDGMENT

We thank Dr. S. Kobayashi and Mrs. K. Hagiwara for their support during this work.

LITERATURE CITED

(1) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **2000**, *130*, 2073S−2085S.

(2) Lee, M. J.; Wang, Z. Y.; Li, H.; Chen, L.; Sun, Y.; Gobbo, S.; Balentine, D. A.; Yang, C. S. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol. Biomarkers Prev.* **1995**, *4*, 393−399.

(3) Nakagawa, K.; Okuda, S.; Miyazawa, T. Dose-dependent incorporation of tea catechins, (−)-epigallocatechin-3-gallate and (−)-epigallocatechin, into human plasma. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1981−1985.

(4) Piskula, M. K.; Terao, J. Accumulation of (−)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rats tissues. *J. Nutr.* **1998**, *128*, 1172−1178.

(5) Miyazawa, T.; Nakagawa, K.; Kudo, M.; Muraishi, K.; Someya, K. Direct intestinal absorption of red fruit anthocyanins, cyaniding-3-glucoside and cyaniding-3,5-diglucoside, into rats and humans. *J. Agric. Food Chem.* **1999**, *47*, 1083−1091.

(6) Matsumoto, H.; Inaba, H.; Kishi, M.; Tominaga, S.; Hirayama, M.; Tsuda, T. Orally administered delphinidin 3-rutinoside and cyaniding 3-rutinoside are directly absorbed in rats and humans and appear in the blood as the intact forms. *J. Agric. Food Chem.* **2001**, *49*, 1546−1551.

(7) Hollman, P. C. H.; van Trijp, J. M. P.; Buysman, M. N. C. P.; van der Gaag, M. S.; Mengelers, M. J. B.; de Vries, J. H. M.; Katan, M. B. Relative bioavailability of the antioxidant flavonoid quercetin from various food in man. *FEBS Lett.* **1997**, *418*, 152−156.

(8) Shimoi, K.; Yoshizumi, K.; Kido, T.; Usui, Y.; Yumoto, T. Absorption and urinary excretion of quercetin, rutin, and αG-rutin, a water soluble flavonoid, in rats. *J. Agric. Food Chem.* **2003**, *51*, 2785−2789.

(9) Shimoi, K.; Okada, H.; Furugori, M.; Goda, T.; Takase, S.; Suzuki, M.; Hara, Y.; Yamamoto, H.; Kinae, N. Intestinal absorption of luteolin and luteolin 7-O-β-gluoside in rats and humans. *FEBS Lett.* **1998**, *438*, 220−224.

(10) Bourne, L. C.; Rice-Evans, C. Bioavailability of ferulic acid. *Biochem. Biophys. Res. Commun.* **1998**, *253*, 222−227.

(11) Olthof, M. R.; Hollman, P. C. H.; Katan, M. B. Chlorogenic acid and caffeic acid are absorbed in humans. *J. Nutr.* **2001**, *131*, 66−71.

(12) Azuma, K.; Ippoushi, K.; Nakayama, M.; Ito, H.; Higashio, H.; Terao, J. Absorption of chlorogenic acid and caffeic acid in rats after oral administration. *J. Agric. Food Chem.* **2000**, *48*, 5496−5500.

(13) Clliford, M. N. Chlorogenic acids and other cinnamates- nature, occurrence and dietary burden. *J. Sci. Food Agric.* **1999**, *79*, 362−372.

(14) Konishi, Y.; Shimizu, M. Transepithelial transport of ferulic acid by monocarboxylic acid transporter in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 856−862.

(15) Konishi, Y.; Kobayashi, S.; Shimizu, M. Transepithelial transport of *p*-coumaric acid and gallic acid by monocarboxylic acid transporter in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 2317−2324.

(16) Konishi, Y.; Hagiwara, K.; Shimizu, M. Transepithelial transport of fluorescein in Caco-2 cell monolayers and its use in *in vitro* evaluation of phenolic acids availability. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 2449−2457.

(17) Kikuzaki, H.; Hisamoto, M.; Hirose, K.; Akiyama, K.; Taniguchi, H. Antioxidant properties of ferulic acid and its related compounds. *J. Agric. Food Chem.* **2002**, *50*, 2161−2168.
(18) Yamada, J.; Tomita, Y. Antimutagenic activity of caffeic acid and related compounds. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 328−329.
(19) Pannala, A. S.; Razaq, R.; Halliwell, B.; Singh, S.; Rice-Evans, C. A. Inhibition of peroxynitrite dependent tyrosine nitration by hydroxycinnamates: nitration or electron donation? *Free Radical Biol. Med.* **1998**, *24*, 594−606.
(20) Li, P.; Wang, H. Z.; Wang, X. Q.; Wu, Y. N. The blocking effect of phenolic acid on *N*-nitrosomorpholine formation *in vitro*. *Biomed. Environ. Sci.* **1994**, *7*, 68−78.
(21) Inoue, M.; Suzuki, R.; Koide, T.; Sakaguchi, N.; Ogihara, Y.; Yabu, Y. Antioxidant, gallic acid, induces apoptosis in HL-60RG cells. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 898−904.
(22) Inoue, M.; Suzuki, R.; Sakaguchi, N.; Li, Z.; Takeda, T.; Ogihara, Y.; Jiang, B. Y.; Chen, Y. Selective induction of cell death in cancer cells by gallic acid. *Biol. Pharm. Bull.* **1995**, *18*, 1526−1530.
(23) Gali, H. U.; Perchellet, E. M.; Klish, D. S.; Johnson, J. M.; Perchellet, J. P. Antitumor-promoting activities of hydrolyzable tannins in mouse skin. *Carcinogenesis* **1992**, *13*, 715−718.
(24) Gali, H. U.; Perchellet, E. M.; Perchellet, J. P. Inhibition of tumor promoter-induced ornithine decarboxylase activity by tannic acid and other polyphenols in mouse epidermis *in vivo*. *Cancer Res.* **1991**, *51*, 2820−2825.
(25) Spencer, J. P. E.; Chowrimootoo, G.; Choudhury, R.; Debnam, E. S.; Srai, S. K.; Rice-Evans, C. The small intestine can both absorb and glucuronidate luminal flavonoid. *FEBS Lett.* **1999**, *458*, 224−230,
(26) Shahrzad, S.; Aoyagi, K.; Winter, A.; Koyama, A.; Bitsch, I. Pharmacokinetics of gallic acid and its relative bioavailability from tea in healthy humans. *J. Nutr.* **2001**, *131*, 1207−1210.
(27) Guo, C.; Cao, G.; Sofic, E.; Prior, R. L. High-performance liquid chromatography coupled with coulometric array detection of electroactive components in fruits and vegetables: relationship to oxygen radical absorbance capacity. *J. Agric. Food Chem.* **1997**, *45*, 1787−1796.
(28) Tsuji, A.; Takanaga, H.; Tamai, I.; Terasaki, T. Transcellular transport of benzoic acid across Caco-2 cells by a pH-dependent and carrier-mediated transport mechanism. *Pharm. Res.* **1994**, *11*, 30−37.
(29) Cong, D.; Fong, A. K.; Lee, R.; Pang, K. S. Absorption of benzoic acid in segmental region of the vascularly perfused rat small intestine preparation *Drug Metab. Dispos.* **2001**, *29*, 1539−1547.
(30) Konishi, Y.; Kubo, K.; Shimizu, M. Structural effects of phenolic acids on the transepithelial transport of fluorescein in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 2014−2017.
(31) Piskula, M. K.; Terao, J. Quercetin's solubility affects its accumulation in rat plasma after oral administration. *J. Agric. Food Chem.* **1998**, *46*, 4313−4317.
(32) Chen, L.; Lee, M. J.; Li, H.; Yang, C. S. Absorption, distribution, and elimination of tea polyphenols in rats. *Drug Metab. Dispos.* **1997**, *25*, 1045−1050.
(33) Chow, H. H.; Cai, Y.; Alberts, D. S.: Hakim, I.; Dorr, R.; Shahi, F.; Crowell, J. A.; Yang, C. S.; Hara, Y. Phase I pharmacokinetics study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenone E. *Cancer Epidemiol. Biomarkers Prev.* **2001**, *10*, 53−58.
(34) Konishi, Y.; Kobayashi, S.; Shimizu, M. Tea polyphenols inhibit the transport of dietary phenolic acids mediated by the monocarboxylic acid transporter (MCT) in intestinal Caco-2 cell monolayers. *J. Agric. Food Chem.* **2003**, *51*, 7296−7302.

Received for review November 20, 2003. Revised manuscript received February 26, 2004. Accepted February 27, 2004.

JF035366K