Phenolic Acids Are Absorbed from the Rat Stomach with Different Absorption Rates

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The intestinal absorption characteristics of phenolic acids (PAs) have been elucidated in terms of their affinity for the monocarboxylic acid transporter (MCT). Recently, the involvement of the stomach has been implicated in the absorption of polyphenols. The present work demonstrates that the gastric absorption efficiency of each PA is apparently different between various PAs. Various PAs with different affinities for MCT were administered (2.25 μmol) to rat stomach, and then the plasma concentration of the PA was measured. The plasma concentration of ferulic acid (FA) peaked 5 min after administration in the stomach. At 5 min after administration, the plasma concentration of each PA increased in the order: gallic acid = chlorogenic acid < caffeic acid < *p*-coumaric acid = FA. This order matches their respective affinity for MCT in Caco-2 cells, which we have demonstrated in previous studies. These results indicated that MCT might be involved in the gastric absorption of PAs, similar to the intestinal absorption.

KEYWORDS: Absorption; monocarboxylic acid transporter; phenolic acid; stomach; rats

INTRODUCTION

The physiological effects of dietary polyphenols on human health have recently received a great deal of attention. Polyphenols in fruit and vegetables are natural antioxidants that are assumed to contribute to the prevention of cardiovascular diseases (*1*). The most abundant types of polyphenol in the human diet are the flavonoids and phenolic acids (PAs). Although many studies have been carried out to investigate the physiological role of flavonoids, much less attention has been focused on PAs. Nevertheless, many common foodstuffs, including fruits, cereals, vegetables, and beverages such as coffee have a high PA content (*2*). Caffeic acid (CA) is the major representative PA and occurs in foods mainly as chlorogenic acid (CLA), an ester of quinic acid. Daily intake of CA or CLA by coffee drinkers is typically ~1 g (*2*). PAs such as CA or CLA exhibit antioxidant activities and anti-mutagenic and anti-carcinogenic effects in vitro (*3*, *4*). Indeed this is consistent with the reported inverse correlation between coffee intake and colon cancer in some epidemiologic studies (*5*−*7*). Therefore, the physiological importance and health effects of PAs on humans should be investigated in detail.

We have recently elucidated the intestinal absorption characteristics of PAs and related compounds (i.e., ferulic acid (FA), *p*-coumaric acid (PCA), gallic acid (GA), CA, CLA, and rosmarinic acid) in terms of their affinity for monocarboxylic acid transporter (MCT). Our studies have demonstrated the diverse nature of intestinal absorption in Caco-2 cells (i.e., MCT-mediated absorption, partial MCT-mediated absorption, paracellular diffusion) (*8*−*11*). The absorption characteristics of these compounds in Caco-2 cells display a good correlation with their absorption efficiency and bioavailability in vivo (*12*, *13*). These findings highlight the unique physiological significance of the MCT-mediated transport system. We have focused on the physiological impact of MCT-mediated absorption and distribution in humans, which involves specific transport systems that act not only for phenolic acids but also for microbial metabolites of poorly absorbed polyphenols or dietary fibers with biological activities, (i.e., "metabo-nutrients") (*14*, *15*).

Recent studies have demonstrated that gastric absorption occurs for some flavonoids such as quercetin, daizein, or anthocyanins (*16*−*18*). Thus, the significance of gastric absorption in terms of the health effects of dietary phytochemicals is becoming an increasingly important issue. This study was designed to clarify the gastric absorption characteristics of various PAs with different affinity for MCT (**Figure 1**) (*8*−*10*) by measuring plasma concentration of PAs dosed in a rat stomach with the use of HPLC-electrochemical detector (ECD) fitted with a Coulometric detection. This report demonstrates an increasing order in the gastric absorption efficiency of various PAs, which matches their respective affinity for MCT in Caco-2 cells previously demonstrated (*8*−*10*). This also suggests that the MCT-mediated absorption system might be involved in the gastric absorption of PAs in rat stomach.

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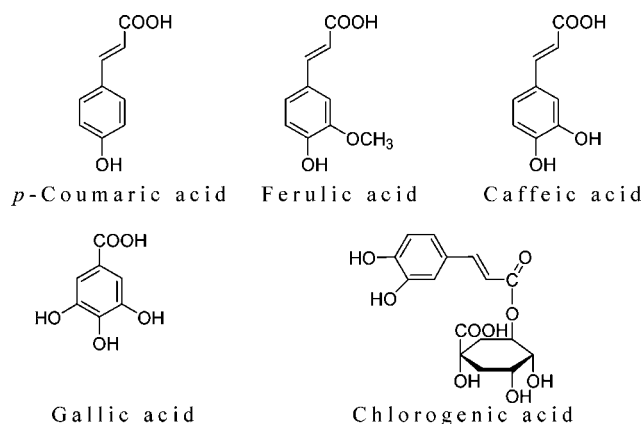† Kirin Brewery Co., Ltd.
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Figure 1. Chemical structures of each PA in rat plasma.

MATERIALS AND METHODS

Animals. Male Wistar rats (8 weeks old) were housed in an air-conditioned room (22 ± 1 °C) under 12 h dark/12 light cycles with free access to tap water and a standard rat diet. The rats were acclimatized to the environment for 4 days before commencement of experiments. The care and treatment of the rats was in accordance with the guidelines prescribed by the Committee for Care of Laboratory Animals in the Graduate School of Agricultural and Life Sciences at the University of Tokyo.

Materials. FA, PCA, GA, CA, CLA, and sulfatase type H-5 were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). The other chemicals used in this study were of analytical grade.

In Situ Gastric Absorption. The protocol for measuring in situ gastric absorption was a modified version of the method described in our previous study (*19*). Briefly, the rats were starved for 5−8 h (to eliminate the influence from the chyme residue in the stomach) and then anesthetized with sodium pentobarbital. The rats were kept alive under anesthesia throughout the experiments. The pylorus was ligated before injecting 2.25 μmol of PAs (FA, PCA, GA, CA or CLA) in 0.5 mL of physiological saline into the stomach at 37 °C, in the same manner as our previous study (*19*). For CA, 1% EtOH was included in the saline to increase solubility. The portal vein and abdominal aorta were then cannulated, and about 0.5 mL of blood was withdrawn from each cannula at 5, 15, and 25 min. Heparin-treated blood was centrifuged at 2000*g* for 15 min at 4 °C to obtain the plasma. The plasma was stored frozen at −30 °C until required for analysis.

Chromatographic Conditions. HPLC-ECD fitted with a coulometric detection system was used for analysis as previously reported (*12, 13*). In brief, to 25 μL of plasma were added 25 μL of 0.1 mol/L sodium acetate buffer (pH 5.0) and 100 μL of 0.83 mol/L acetic acid in methanol. The mixture was vortexed, sonicated, and centrifuged (at 8500*g* for 5 min at 4 °C), and the supernatant was injected onto an HPLC C18 column (ODS150, MC Medical, Inc., Tokyo, Japan). For FA and PCA, mobile phase A (solvent A) was 50 mM sodium acetate containing 5% methanol (pH 3.0), and mobile phase B (solvent B) was 50 mM sodium acetate containing 40% acetonitrile and 20% methanol (pH 3.5). 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. For CA, CLA, and 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. Eight electrode detector potentials (0−700 mV in increments of 100 mV) were used to measure all of PAs. Conjugated CA in the portal vein and conjugate CLA both in the portal vein and abdominal artery were not detected in this experiment.

Enzymatic Hydrolysis and Determination of Each PA Conjugate. Plasma (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 about 270 units of β-glucuronidase activity. The mixture was incubated at 37 °C for 45 min. The difference in each PA content before and after sulfatase treatment was assumed to be due the amount of the respective sulfate and glucuronide conjugates in the sample (conjugated PA), as shown previously (*12, 13*). Each PA detected with and without the sulfatase treatment are presumed to be total and intact PA, respectively.

Statistical Analysis. Data are shown as mean ± SEM ($n = 3$ or more). Tukey's multiple-range test was used when significant differences were obtained by one-way ANOVA. Significance was set at $P < 0.05$.

RESULTS

Determination of Each PA Absorbed in Plasma. Detection limit for all PAs is <0.5 pmol on the column, and purity of the peaks was assessed using peak area ratio accuracies for the adjacent oxidation channels (lower or upper) to the dominant oxidation channel (>70% ratio accuracy) (*20*). **Figure 2** shows representative HPLC profiles of plasma dosed each PA. The retention time (RT) and dominant oxidation potential for PCA, FA, GA, CA, and CLA were 14.6 min and 600 mV, 16.0 min and 500 mV, 6.7 min and 100 mV, 18.7 min and 100 mV, and 17.6 min and 200 mV, respectively.

Quantitative Changes in FA and Its Metabolites in Rat Plasma. Concentration changes of FA and its metabolites in the portal vein and abdominal artery as a function of time after administration are shown in **Figure 2**. The gastric absorption of intact FA initially peaked at 5 min and decreased rapidly both in the portal vein and in the abdominal artery (35.1 μmol/L for the portal vein, and 2.4 μmol/L for the abdominal artery). By contrast, no drastic changes in conjugated FA with time were observed in either the portal vein or the abdominal artery (13.0−16.8 μmol/L for the portal vein; 11.3−17.0 μmol/L for the abdominal artery).

Comparison of Each PA Concentration in the Portal Vein and Abdominal Artery at 5 min after Administration. At 5 min after administration, the concentration of each PA and their respective conjugates in the portal vein and the abdominal artery were examined (**Figure 3**). The concentration of each intact PA in the portal vein and the abdominal artery, respectively, were as follows: 23.78 and 8.92 μmol/L for PCA, 13.02 and 1.76 μmol/L for FA, 1.88 and 0.36 μmol/L for CA, 0.45 and 0.05 μmol/L for GA, and 0.12 and 0.04 μmol/L for CLA. In contrast, the concentration of each conjugated PA in the portal vein and the abdominal artery, respectively, were as follows; 9.2 and 7.1 μmol/L for PCA, 6.9 and 7.6 μmol/L for FA, 0.51 μmol/L (abdominal artery) for CA, and 0.09 and 0.16 μmol/L for GA.

DISCUSSION

Previously we administered 2.25 μmol of FA into the rat stomach and examined the recovery of FA and corresponding metabolites after 25 min incubation in situ in the gastric content, mucosa, portal vein, celiac arterial plasma, bile, and urine (*19*). We anticipated FA to be quickly absorbed intact from the rat stomach and then metabolized mainly in the liver (*19*). In this study, we have improved the sensitivity and accuracy of the measurement for each PA by employing a Coulometric detection systems using HPLC-ECD. We investigated changes in the concentration of FA and the corresponding metabolites over time in the portal vein and the abdominal artery for up to 25 min after administration under the same experimental conditions used in our previous study (*19*). As shown in **Figure 2**, the concentration of intact FA peaked at 5 min and then decreased

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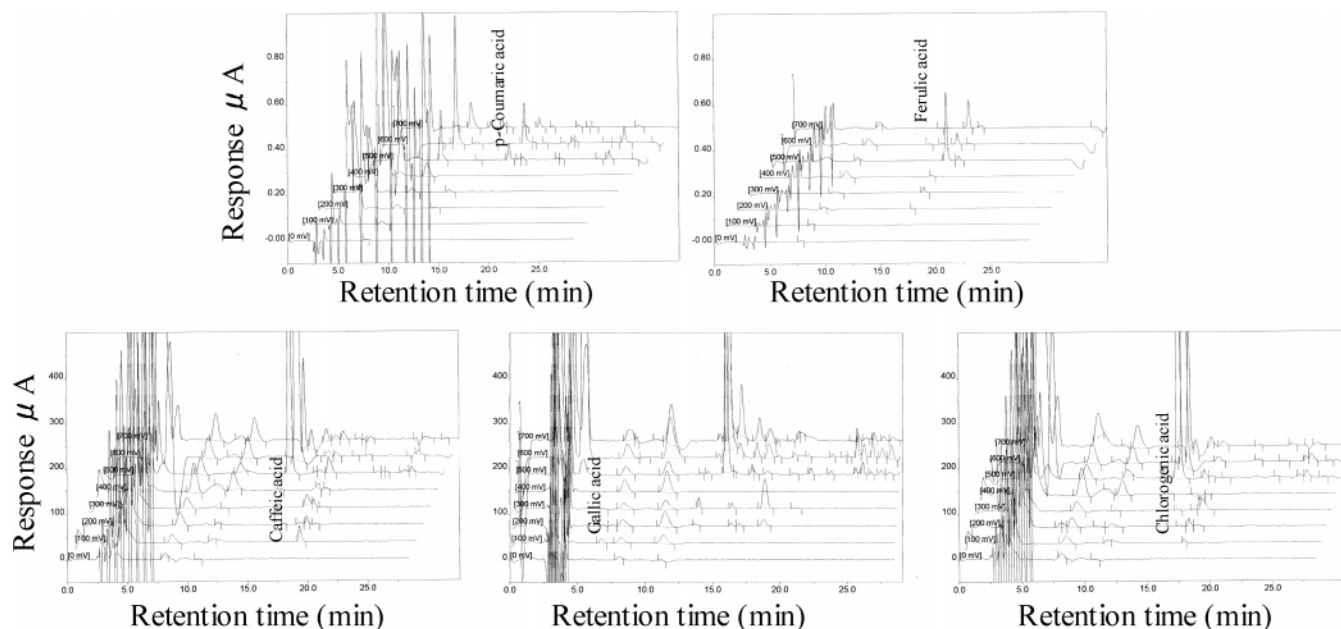

Figure 2. Chromatograms obtained by HPLC-ECD analysis of rat plasma administered each PA.
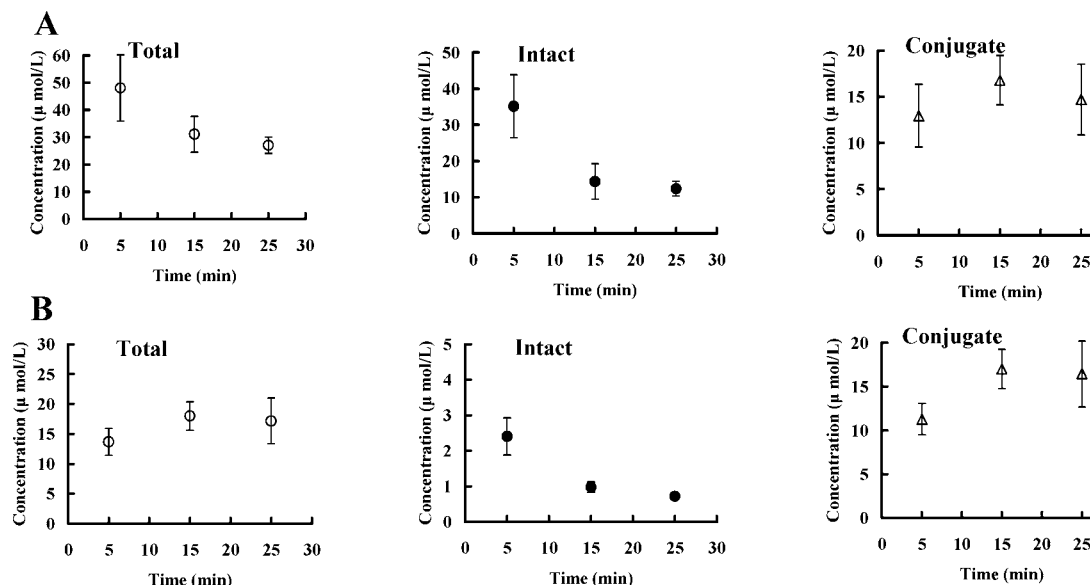

Figure 3. Plasma concentration profiles of FA in the portal vein (**A**) and the abdominal artery (**B**). Each point is the mean ± SEM, $n = 3$ or more.

rapidly in both the portal vein and the abdominal artery. However, there was a slow increase in the level of conjugated FA in both the portal vein and the abdominal artery (peak level; 15 min). These results suggest that there might be two different systems for the gastric absorption of FA: a rapid permeation system for intact FA and a slow permeation system for the conjugated derivatives. In our previous study, however, conjugated FA was not detected in the gastric content or mucosa. Furthermore, the proportion of free FA to total FA in bile was as low as that in arterial plasma, although the proportion of free FA to total FA in the portal vein was very high (49%) (*19*). These observations suggest that the conjugated FA detected in the portal vein and abdominal artery were derived from metabolism in the liver and/or re-absorption by enterohepatic circulation.

To examine the efficiency of gastric absorption for each PA, we determined the plasma concentration of various PAs and their metabolites at 5 min after administration (**Figure 4**). The concentration of each intact PA in both the portal vein and the

abdominal artery increased in the order: CLA = GA < CA < FA < PCA. These results are in good agreement not only with the order observed in the absorption efficiency and the bioavailability in vivo (GA < CA < PCA) (*12, 13*) but also with the affinity order for MCT in Caco-2 cells (CLA = GA < CA < FA = PCA) (*8−10*). Overall, our results indicate that the MCT-mediated absorption system is involved in gastric absorption of PA, as demonstrated previously for intestinal absorption.

The ratio of the concentration of intact PCA and FA in the abdominal artery to that in the portal vein was 0.38 and 0.14, respectively. These results indicate that FA is likely to be more susceptible to hepatic elimination than PCA. This is consistent with our previous study, which established that FA was mainly metabolized in the liver (*19*). It is generally thought that the first hepatic elimination of hydrophobic compounds is quite efficient. Xenobiotic compounds with an affinity for biomembranes are likely to be harmful because they could readily permeate across the epithelium and enter the enterocytes. It is possible that an unknown but specific elimination mechanism
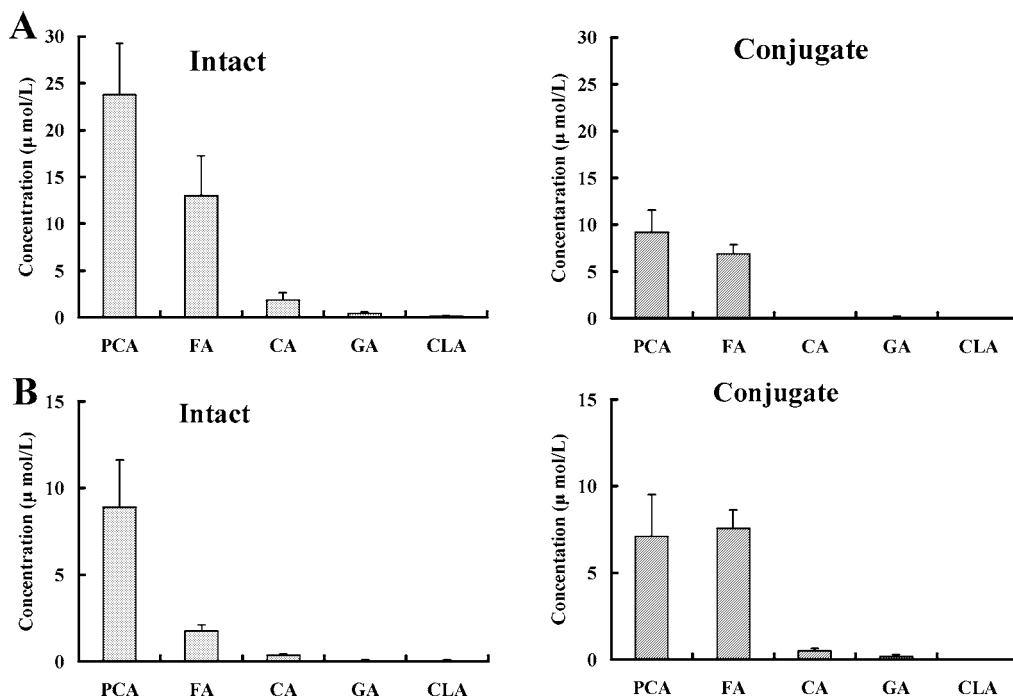
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Figure 4. Plasma concentration of each PA in the portal vein (**A**) and the abdominal artery (**B**) at 5 min after administration. Each point is the mean ± SEM, $n = 3$ or more.

Table 1. Percentage of Concentration Intact PAs to That of Total PAs in the Plasma of Portal Vein and Abdominal Artery at 5 min after Injection of PA (2.25 μmol) into Stomach[a]

PAs	portal vein %	abdominal artery %
PCA	73.3 ± 3.6a	57.5 ± 4.4a
FA	62.8 ± 4.9a	18.4 ± 1.5b
CA	89.7 ± 16.9a	39.1 ± 0.5c

[a] Values are the mean ± SEM of three experiments. Mean in a column without a common letter differ, $P < 0.05$.

preserves homeostasis. The initial step in the elimination of a xenobiotic, such as PA, is the conjugation of the intact compound with sulfate and/or glucuronide in the gastrointestinal mucosa and/or liver after absorption (*21*, *22*). In this study, the proportion of intact FA to total FA declined rapidly after the absorbed FA from the stomach passed through the liver (62.8 ± 4.9% in the portal vein vs 18.4 ± 1.5% in the abdominal artery; **Table 1**). These results indicate that the intact FA is efficiently conjugated in the liver (*19*). Compared with that of FA, the conjugation of PCA and CA appears to occur more slowly (**Table 1**). The 3-methoxy moiety in FA (**Figure 1**) is presumably a good target for conjugation enzymes such as UDP-glucuronosyltransferases and sulfotransferases. The slower conjugation of PCA and CA as compared to FA may explain the differences in the rate of elimination (this study and ref 21 vs refs 12 and 22).

To date, 14 isoforms of MCT have been identified (*23*), although only MCT1 to MCT4 are characterized in terms of their substrate and inhibitor kinetics. Each MCT isoform is likely to have a unique biological role, which is related to the different tissue distribution. MCT1, the best characterized isoform, is found in almost all tissues of the human body (*24*), which would imply a physiological significance for humans. Furthermore, MCT1 has been reported to be expressed in the stomach of mouse, hamster, and calf (*25*−*27*). In contrast, it is also possible that MCT subtypes other than MCT1−MCT4 may participate in the gastric absorption of each PA, as in the case for the

intestinal absorption. Actually, we have recently demonstrated that MCT1 has not participated in the transport of PA by the analysis with MCT1 expressing Caco-2 cells and oocytes (*28*). It was reported that various subtypes of MCT, such as MCT1, MCT3, MCT4, MCT5, and MCT6, are expressed in Caco-2 cells (*29*). Furthermore, it was recently reported that different subtypes of MCTs participate in the absorption of PA in Caco-2 cells: MCT on the apical side appears to be different from MCT on the basolateral side in terms of the affinity for Artepillin C (*30*). Further studies are required to establish the MCT subtype responsible for gastric and intestinal absorption of PA. Furthermore, tissue distribution and subcellular localization of the relevant MCT subtype will have to be determined to fully assess the health effects of both PA and the corresponding "metabonutrients" (*15*).

In conclusion, we have demonstrated that each PA is absorbed from the rat stomach with different absorption efficiency, which matches their respective affinity for MCT in Caco-2 cells previously demonstrated (*8*−*10*). Furthermore, this suggests that the MCT-mediated absorption system might be involved in the gastric absorption of PAs, as is the case with the intestinal absorption. Our findings highlight the physiological significance of MCT-mediated transport system for bioactive components of the diet.

ABBREVIATIONS USED

PA, phenolic acid; PCA, *p*-coumaric acid; FA, ferulic acid; GA, gallic acid; CA, caffeic acid; CLA, chlorogenic acid; MCT, monocarboxylic acid transporter; ECD, electrochemical detector.

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