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Pharmacokinetic Study of Caffeic and Rosmarinic Acids in Rats after Oral Administration

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p-Coumaric and ferulic acid are actively taken up by monocarboxylic acid transporter (MCT), whereas gallic acid, caffeic acid (CA), and rosmarinic acid (RA) are absorbed by paracellular diffusion in human intestinal Caco-2 cells, although CA has low affinity for MCT. We previously demonstrated that *p*-coumaric acid has a much higher absorption efficiency than gallic acid in rats, owing to the MCT-mediated absorption of *p*-coumaric acid *in vivo* (*J. Agric. Food Chem.* **2004**, *52*, 2527−2532). Here, absorption of orally administered CA and RA in rats has been studied to investigate their intestinal absorption characteristics and pharmacokinetics *in vivo* and to compare the results with those of *p*-coumaric and gallic acids obtained under identical conditions. Rats were given 100 μmol/kg body weight of CA and RA, and blood was collected from the portal vein and abdominal artery after administration. CA, RA, and their metabolites were quantified by a coulometric detection method using HPLC−ECD. The serum concentration of intact CA and RA in the portal vein peaked at 10 min after administration, with a C_{max} of 11.24 μmol/L for CA and 1.36 μmol/L for RA. The area under the curve (AUC) for intact CA and RA in the portal vein was calculated from the serum concentration−time profile to be 585.0 and 60.4 μmol min L^{-1}, respectively. The absorption efficiency of CA was about 9.7-fold higher than that of RA. Overall, the absorption efficiency of these compounds *in vivo* increases in the order: gallic acid = RA < CA < *p*-coumaric acid, which is in good agreement with results obtained in Caco-2 cells *in vitro*.

KEYWORDS: Caffeic acid; rosmarinic acid; monocarboxylic acid transporter; intestinal absorption; rats

INTRODUCTION

Dietary polyphenols are widely assumed to be beneficial to human health by exerting various biological effects. Polyphenols are widely distributed in edible plants and are classified into phenolic acids, flavonoids, and the less common stilbenes, lignans, lignins, and coumarins. The potential benefit of flavonoids in nutrition has attracted much research interest over the past decade. Many studies have focused on their absorption and metabolism to determine both their bioactive compounds *in vivo* and the mechanisms by which they might exert physiological effects. Various metabolites of flavonoids have been detected in the plasma and urine (*1*−*9*). The first limiting step in the ingestion of flavonoids must be their intestinal absorption. The absorption efficiency of polyphenols is generally low, however, and the compounds that are bioactive *in vivo* are still unknown (*10*−*13*). Colonic microbial metabolites of flavonoids have been detected in high abundance in the urine and plasma and have being proposed to play a putative role as bioactive components of their parent flavonoids *in vivo* (*14*−*18*).

Unlike flavonoids, phenolic acids have not been extensively studied and are not considered to be of great nutritional interest. Phenolic acids are present in many foods including grains, vegetables, and fruits (*19*). It has been shown that certain phenolic acids, such as ferulic or *p*-coumaric acids, are actively absorbed by the monocarboxylic acid transporter (MCT) in Caco-2 cells (*20*, *21*). However, dihydroxy and trihydroxy derivatives of benzoic or cinnamic acids, such as caffeic acid (CA) and gallic acid, have low or no affinity for MCT and are mainly absorbed by paracellular diffusion (*21*−*23*). Similarly, esterified phenolic acids, such as chlorogenic acid and rosmarinic acid (RA), also have no affinity for MCT and are absorbed by paracellular diffusion, because the ester group might negatively affect interactions with MCT (*22*, *24*). Indeed, the relative oral bioavailability of *p*-coumaric acid to gallic acid is ∼70 in rats. This significant difference in bioavailability

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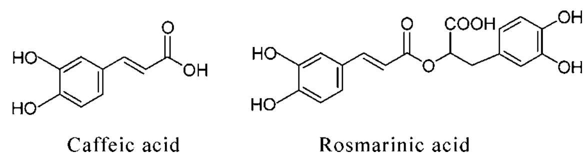

Figure 1. Chemical structures of CA and RA.

illustrates the high absorption efficiency provided by MCT-mediated transport *in vivo* (*25*).

The finding that phenolic acids are rapidly absorbed and distributed intact within the body has focused significant research effort toward determining the nutritional value of these compounds. Phenolic acids such as CA and RA (**Figure 1**) are antioxidants (*26*, *27*) with antimutagenic, anticarcinogenic, antiinflammatory, and antiallergenic activities (*28*−*33*). Commercial materials containing CA or RA have been manufactured and are available for specific use in anticipation of their biological activity. To date, however, several studies have described the absorption and metabolism of these two compounds (*14*, *34*−*41*), but the absorption characteristics and pharmacokinetic profiles of CA and RA have been scarcely examined in detail.

This study was designed to investigate the intestinal absorption characteristics, bioavailability, and pharmacokinetics of CA and RA *in vivo* and to compare them with those of *p*-coumaric acid and gallic acid, which have been previously determined under identical conditions (*25*).

MATERIALS AND METHODS

Materials. CA, RA, 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.

Animals and Diets. Male Wistar rats (6 weeks old, Charles River Japan, Yokohama, Japan) were housed in an air-conditioned room (22 ± 1 °C) under 12 h dark/12 h light cycles, with free access to tap water and commercial nonpurified CE-2 diet (CLEA Japan, Inc., Tokyo, Japan). Three rats administered CA or RA were assigned to each time point of each experimental group. This study was approved by the Ethics Committee of Kirin Brewery Co., Ltd.

Sample Preparation. Rats were fasted for 20 h, and their body weight was measured (147−177 g). They were given CA or RA (100 μmol/kg in 10% propyleneglycol) by gastric intubation. Blood was withdrawn from the portal vein and abdominal artery at each time point (5, 10, 20, 30, 60, and 90 min) after administration of CA or RA. Serum was obtained by centrifugation and was stored at −80 °C until analysis.

HPLC−ECD Analysis. An HPLC−ECD fitted with a coulometric detection system was used to measure the amount of CA, RA, and their conjugates in serum samples according to a previously described method (*25*). In brief, to 25 μL of serum 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). The mobile phase A (solvent A) was 50 mmol/L sodium acetate containing 5% methanol (pH 3.0 adjusted with phosphoric acid), while the mobile phase B (solvent B) was 50 mmol/L sodium acetate containing 40% acetonitrile and 20% methanol (pH 3.5 adjusted with phosphoric acid). We used the following elution profile (0.6 mL/min): 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 with 0% solvent A/100% solvent B; and 31−35 min, isocratic elution with 85% solvent A/15% solvent B. The eight electrode detector potentials were increased from 0 to 700 mV in increments of 100 mV. Quantitative determination of CA and RA was performed by using an external standard method, which verified that the detector response was linear for a concentration of up to 400 μmol/L for CA and 500 μmol/L for RA.

Enzymatic Hydrolysis and Determination of CA or RA 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 about 270 units of β-glucuronidase activity. The mixture was incubated at 37 °C for 45 min. The difference in CA or RA content before and after sulfatase treatment was assumed to be due to the amount of the respective sulfate and glucuronide conjugates in the sample.

Measurement of the Partition Coefficient in an Octanol/Water Mixture. To determine the partition coefficient, a sample solution (200 μmol/L) in *n*-octanol was kept at 40 °C for 1 h and the absorbance at 254 nm was measured (A_0). An equal volume of phosphate-buffered saline was added, and the mixture was vortexed, maintained at 40 °C for 1 h, and then centrifuged at 210*g* for 10 min, before the absorbance of the organic phase was measured (A_s) at 254 nm. The partition coefficient was calculated as A_s/A_0.

Data Analysis. Noncompartmental pharmacokinetic parameters were calculated from the serum concentration−time data by using WinNonlin. The measured values were used for the maximum serum concentration, C_{max}, and the time to reach the maximum serum concentration, t_{max}. The results of C_{max} are expressed as mean ± SEM of three determinations. The area under the curve (AUC) for the serum concentration−time data from zero to the final sampling time at 1.5 h ($AUC_{0-1.5\ h}$) was calculated by using the linear/log trapezoidal rule. Elimination half-life ($t_{1/2}$) was calculated from log-linear regression of the terminal phase of the serum concentration−time profile. AUC and $t_{1/2}$ were calculated by using of mean concentration value in each time point.

RESULTS

Determination of CA and RA in Serum Samples. Figure 2 shows representative HPLC profiles of serum from a control rat (A) and serum from rats given CA (B) and RA (C). On the basis of a comparison in two dimensions (i.e., chromatographic and voltammetric), the identity of the CA or RA peak was determined by evaluating the peak area ratio for the oxidation channels (lower or upper) adjacent to the dominant oxidation channel. An accuracy in the ratio of more than 70% was considered to support peak purity (*42*). The retention time (RT) and dominant oxidation potential for CA and RA were 10.9 min and 200 mV and 19.9 min and 200 mV, respectively. Experiments with CA- or RA-spiked serum showed that this procedure gave more than 97% recovery for both compounds throughout the detection range.

Quantitative Changes in CA, RA, and Their Metabolites in Rat Serum. The mean serum concentration−time profiles of CA, RA, and their metabolites in the portal vein after administration are shown in **Figure 3**. Total and intact concentrations of CA and RA were measured after and before deconjugation with sulfatase treatment, and the results of the noncompartmental pharmacokinetics analysis are given in **Table 1**. The intestinal absorption of intact CA and RA was fast. There was a difference in the AUC in the portal vein (AUC_{portal}) calculated for intact CA and RA. The absorption efficiency of CA was estimated to be 9.7-fold greater than that of RA. This result shows that the intestinal absorption efficiency of CA is higher than that of RA (**Table 1**).

Furthermore, the concentration profiles of CA, RA, and their metabolites in serum in the abdominal artery were investigated to clarify hepatic elimination (**Figure 4**), and the results of the noncompartmental pharmacokinetics analysis are given in **Table 2**. Intact CA peaked at 10 min after administration and decreased rapidly, similar to what was observed in the portal vein. A similar trend was also observed for intact RA. Intriguingly, the AUC in the abdominal artery ($AUC_{abdominal}$) for CA was about 6.6-fold greater than that for RA (**Table 2**).

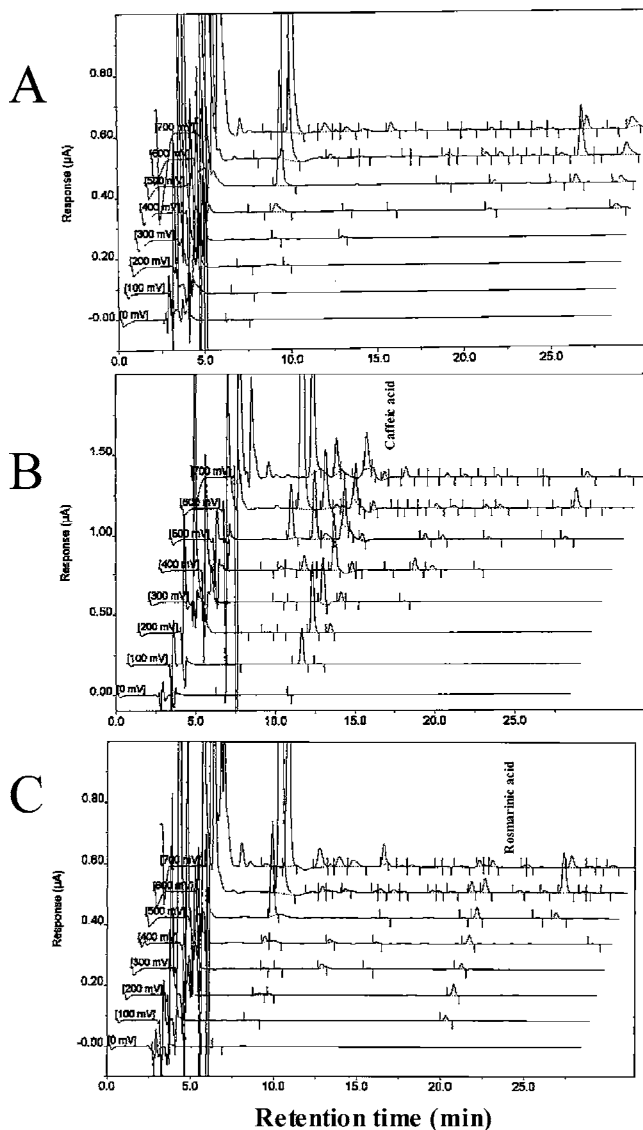

Figure 2. Chromatograms obtained by HPLC–ECD analysis of rat serum before (A) and after the administration of CA (B) and RA (C).

The concentration profiles of conjugated CA and RA in the portal vein are similar to those in the abdominal artery (**Figures 3** and **4**), and the noncompartmental pharmacokinetics analysis of conjugated CA and RA was done and shown in **Table 3**. The values of AUC and C_{max} of conjugated CA and RA in the portal vein are nearly the same as those in the abdominal artery, although the t_{max} values for conjugated CA and RA were later than the t_{max} values for intact CA and RA, respectively (**Table 3**).

Partition Coefficient of CA and RA. To examine the lipophilicity, CA and RA in addition to *p*-coumaric acid and gallic acid were subjected to partition between *n*-octanol and water (**Table 4**). The value of the partition coefficient increased in the order: gallic acid < CA < RA < *p*-coumaric acid, indicating that the order of the solubility of these compounds in the gastrointestinal tract is likely to be reversed.

DISCUSSION

In this study, we have demonstrated that the absorption efficiency of CA was higher than that of RA *in vivo*, indicating indeed that there is a specific difference in the absorption characteristics of the two compounds (**Figure 3** and **Table 1**). The solubility of ingested flavonoids has been reported to increase absorption significantly, because a decrease in precipitation of the compounds in the alimentary tract was found to raise the amount available for absorption (*7*, *8*, *43*). In our previous *in vivo* study, we demonstrated that *p*-coumaric acid was absorbed much more efficiently than gallic acid (AUC_{portal} for *p*-coumaric acid, 2991.3 μmol min L^{-1}; AUC_{portal} for gallic acid, 42.6 μmol min L^{-1}) (*25*), although the solubility of *p*-coumaric acid is lower than that of gallic acid. In this study, we measured the partition coefficient of CA and RA together with those of *p*-coumaric and gallic acids (**Table 4**). The values of the partition coefficient of CA, *p*-coumaric acid, and gallic acid were almost consistent with the previously reported values (CA, 0.08; *p*-coumaric acid, 0.22; gallic acid, 0.02) (*26*). The lipophilicity increases in the order: gallic acid < CA < RA < *p*-coumaric acid, indicating that gallic acid is the most soluble of these compounds. However, the absorption efficiency of these compounds *in vivo* is not related to their solubility in the gastrointestinal tract. Indeed, *p*-coumaric acid and CA were absorbed better (AUC_{portal} for *p*-coumaric acid, 2991.3 μmol min L^{-1}; AUC_{portal} for CA, 585.0 μmol min L^{-1}), whereas the absorption efficiency of RA *in vivo* was nearly as low as that of gallic acid (AUC_{portal} for RA, 60.4 μmol min L^{-1}; AUC_{portal} for gallic acid, 42.6 μmol min L^{-1}).

In a previous *in vitro* study using Caco-2 cells, we demonstrated that the main absorption pathway for CA and RA is paracellular diffusion, although CA is transported by MCT to some extent (*22*, *24*). A monoanionic carboxyl group and a nonpolar side chain or aromatic hydrophobic moiety are considered to be structural criteria for MCT substrates (*44*). Thus, the affinity of phenolic acids for MCT could depend on their structure. Hydroxylation of the MCT substrate, such as in benzoic and cinnamic acids, would decrease the affinity of these acids for MCT because hydrogen bonding between the hydroxyl group of the substrate and MCT might interfere with the molecular recognition (*23*). Moreover, esterified phenolic acids, such as RA or chlorogenic acid, have no affinity for MCT because the carboxylic ester group is likely to interfere with recognition of the monocarboxylic anion group of the substrate (*22*, *24*). The affinity of substrates for MCT is considered to increase in the order: gallic acid = RA = chlorogenic acid < CA < *p*-coumaric acid. Therefore, the difference in absorption efficiency between CA and RA in Caco-2 cells is likely to be due to obvious differences in the way in which they are absorbed, namely, partial MCT-mediated absorption for CA versus paracellular diffusion for RA (*22*, *24*). The results obtained in this study also indicate that there are differences in the absorption characteristics of CA and RA and that a specific mechanism is involved in the absorption of CA *in vivo*, as was found for *p*-coumaric acid (*25*). Furthermore, the absorption efficiency *in vivo* increases in the order: gallic acid = RA < CA < *p*-coumaric acid; this order matches both the affinity of these compounds for MCT and the absorption efficiency obtained in the *in vitro* Caco-2 cell system (*21*, *22*, *24*).

A considerable amount of CA and RA conjugates was observed in the portal vein (**Figure 3**), although no CA and RA conjugates were transported in Caco-2 cells (*22*, *24*). This finding indicates that conjugation of CA and RA occur during their permeation across the rat epithelium, consistent with previous results obtained by an *in situ* perfusion analysis (*37*). Similar results were obtained for *p*-coumaric and gallic acids *in vivo* (*25*). The discrepancy in conjugate formation between the *in vivo* or *in situ* perfusion analysis and the *in vitro* Caco-2 cell system might originate from differences in the evaluation

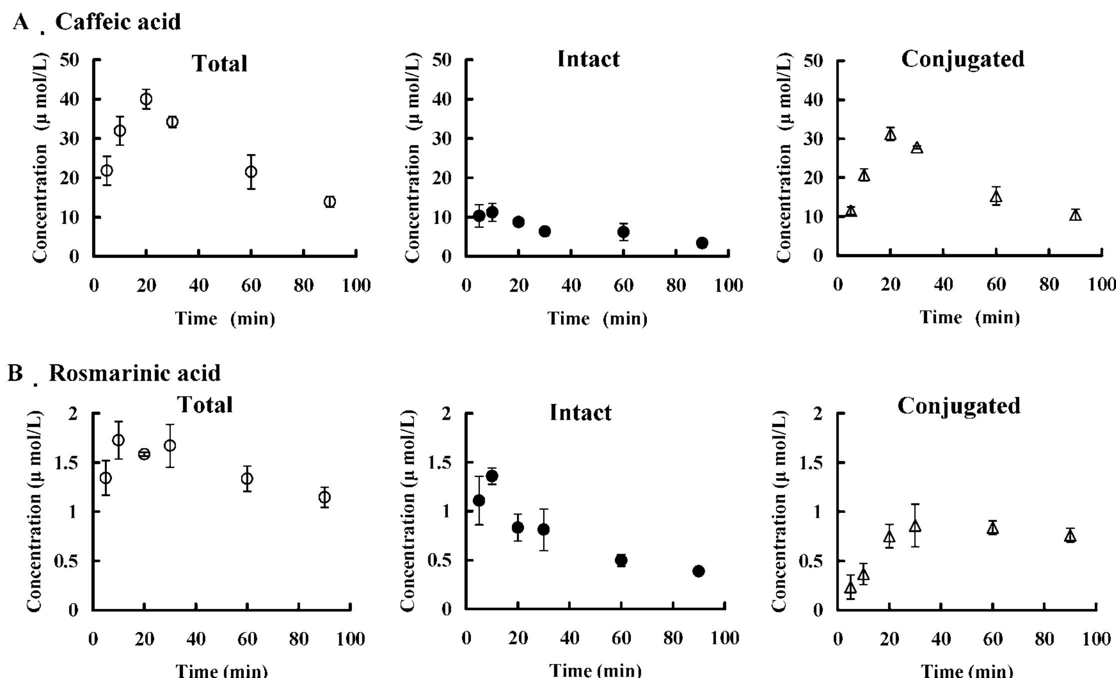

Figure 3. Serum concentration–time profiles of phenolic acid in the portal vein after the administration of CA (A) and RA (B). Each point is expressed as the mean ± SEM, $n = 3$.

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

	CA	RA
C_{max}[b] (µmol/L)	11.24 ± 2.29	1.36 ± 0.08
t_{max} (min)	10	10
$t_{1/2}$ (min)	34.8	56.9
$AUC_{0-1.5\ h}$ (µmol min L^{-1})	585.0	60.4
relative absorption efficiency	9.7	

[a] Values of C_{max} are the mean ± SEM, $n = 3$. [b] Abbreviation: C_{max}, maximum serum concentration; t_{max}, time to reach the C_{max}; AUC, area under the serum concentration–time curve; $t_{1/2}$, elimination half-life. Relative absorption efficiency was calculated as follows: AUC of CA/AUC of RA.

method employed. Because the AUC_{portal} of conjugated CA and RA was nearly the same as the $AUC_{abdominal}$ of conjugated CA and RA (**Table 3**), it is possible that CA and RA were conjugated mainly during the absorption process and that further conjugation had not occurred in the liver (**Figures 3** and **4** and **Table 3**), although t_{max} for conjugated CA and RA were later than t_{max} for intact CA and RA, respectively. It would be speculated that the differences in concentration profiles of intact and conjugated CA or RA might ascribe to the lag time in absorption site and conjugation site *in vivo*, but the exact reason is unknown. In contrast, at 10 min after administration, the concentrations of intact CA and RA in the portal vein were higher than those in the abdominal artery (11.24 versus 2.27 µmol/L for CA and 1.36 versus 0.34 µmol/L for RA). This observation indicates that intact CA and RA were substantially eliminated in the liver. The ratio of $AUC_{abdominal}$ to AUC_{portal} was 0.19 for CA and 0.27 for RA, respectively, which suggests that intact CA is more susceptible to hepatic elimination rather than is intact RA. We previously reported that intact and conjugated *p*-coumaric acid are eliminated similarly in the liver, whereas intact and conjugated gallic acid are scarcely eliminated (*25*). There may be the difference in elimination characteristics in the liver, as was observed in the intestinal absorption. Considering the ratio (0.57) of $AUC_{abdominal}$ (1703.8 µmol min L^{-1}) to AUC_{portal} (2991.3 µmol min L^{-1}) for *p*-coumaric acid, *p*-coumaric acid is less susceptible to hepatic elimination and is more likely to be distributed in the body than CA and RA.

There have been several studies on the absorption and metabolism of CA and RA (*14*, *34*−*41*). Azuma and co-workers reported that, after oral administration of CA or chlorogenic acid (700 µmol/kg body), the concentration of intact CA in the tail vein was at most ∼1 µmol/L during the experimental period (∼6 h) and that CA was present mainly as glucuronide (∼40 µmol/L) (*36*). In contrast, intact chlorogenic acid was not detected during the experimental period, indicating that CA has a higher absorption efficiency than chlorogenic acid. It has been also reported that CA is absorbed better than chlorogenic acid in rats and that the plasma concentration of total CA (intact and conjugated) was 41.3 µmol/L after 8 days of intake (*14*). Although dosing amounts of CA differed, these results are in keeping with our study, in which the concentrations of intact CA and conjugated CA were at most ∼2 and ∼30 µmol/L, respectively.

On the other hand, Nakazawa and Ohsawa have studied the metabolism of orally administered RA or *Perilla frutescens* extract containing RA in rats and humans (*38*, *39*). The recovery of intact RA in the urine was 0.077% of the amount ingested, indicating that the absorption of RA was quite low (*38*). A low absorption of RA was also found in rats and humans given RA (*40*, *41*). The level of total RA (intact and conjugated) in the femoral artery reached a maximum concentration of 4.6 µmol/L at 0.5 h after oral administration in rats (139 µmol/kg body), and the total recovery of RA in urine was 0.44% (*40*). Our present results is well-consistent with these results.

It is generally assumed that the biological activity is highly dependent on the structure of the compound; in particular, the smaller presence of hydroxyl groups and the conjugation of parent compounds (glucuronidation or sulfation) are presumed to decrease bioactivity. Therefore, the bioactivity of *p*-coumaric acid *in vitro* is likely to be lower than that of the other compounds. It is now clear, however, that the ultimate antioxidant potential and, indeed, the resulting bioactivity *in vivo* are

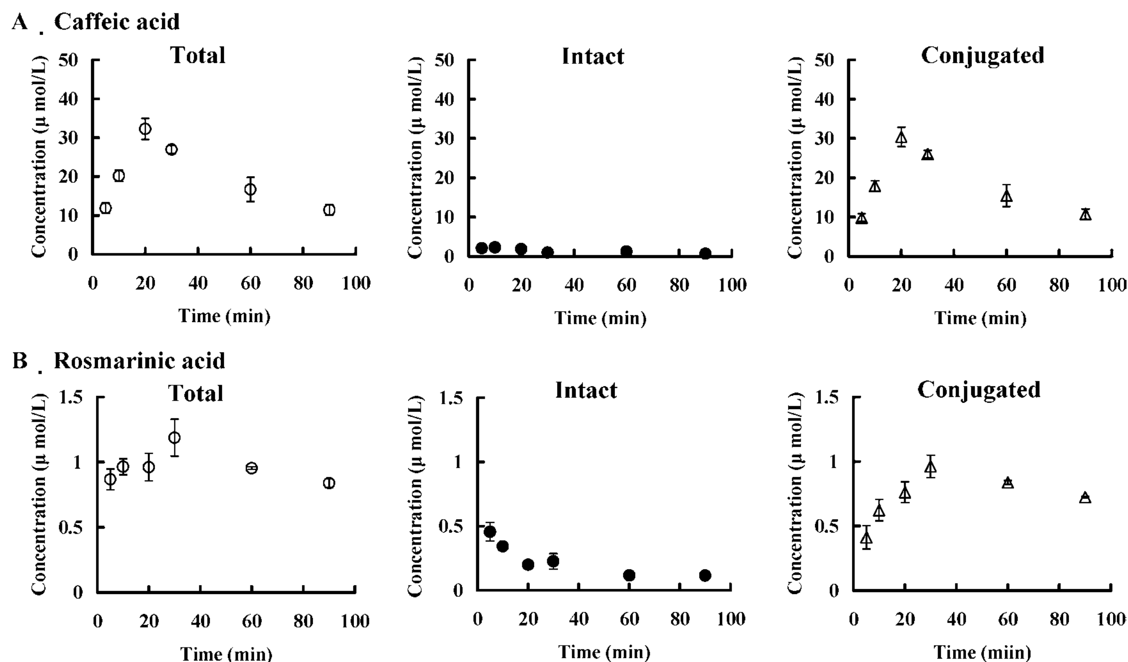

Figure 4. Serum concentration–time profiles of phenolic acid in the abdominal artery after the administration of CA (A) and RA (B). Each point is expressed as the mean ± SEM, $n = 3$.

Table 2. Pharmacokinetic Parameters of Intact RA and CA in the Abdominal Artery after the Administration of a Single 100 μmol/kg Oral Dose of RA and CA[a]

	CA	RA
C_{max}[b] (μmol/L)	2.27 ± 0.16	0.46 ± 0.07
t_{max} (min)	10	5
$t_{1/2}$ (min)	34.3	63.9
$AUC_{0-1.5\ h}$ (μmol min L^{-1})	109.7	16.6
relative bioavailability	6.6	

[a] Values of C_{max} are the mean ± SEM, $n = 3$. [b] Abbreviation: C_{max}, maximum serum concentration; t_{max}, time to reach the C_{max}; AUC, area under the serum concentration–time curve; $t_{1/2}$, elimination half-life. Relative bioavailability was calculated as follows: AUC of CA/AUC of RA.

Table 3. Pharmacokinetic Parameters of Conjugated CA and RA in the Portal Vein and Abdominal Artery after the Administration of a Single 100 μmol/kg Oral Dose of CA and RA[a]

	CA	RA
portal vein		
C_{max}[b] (μmol/L)	31.23 ± 1.69	0.86 ± 0.22
t_{max} (min)	20	30
$AUC_{0-1.5\ h}$ (μmol min L^{-1})	1673.4	65.2
abdominal artery		
C_{max} (μmol/L)	30.39 ± 2.48	0.96 ± 0.09
t_{max} (min)	20	30
$AUC_{0-1.5\ h}$ (μmol min L^{-1})	1614.4	69.5

[a] Values of C_{max} are the mean ± SEM, $n = 3$. [b] Abbreviation: C_{max}, maximum serum concentration; t_{max}, time to reach the C_{max}; AUC, area under the serum concentration–time curve.

dependent on the absorption, metabolism, and distribution of the compound within the body after ingestion. The greater absorption and/or distribution of *p*-coumaric acid is likely to have a significant impact on human health. The biological properties of phenolic acids, which are considered to be efficiently absorbed and distributed by MCT, must be studied in more detail. Furthermore, we previously demonstrated that hydroxylated phenylpropionic or phenylacetic acids, which are

Table 4. Partition Coefficients of CA, RA, *p*-Coumaric Acid, and Gallic Acid in an Octanol/Water Mixture[a]

compound	partition coefficient
CA	0.14 ± 0.01
RA	0.21 ± 0.01
p-coumaric acid	0.24 ± 0.01
gallic acid	0.06 ± 0.01

[a] Values are the mean ± SEM of four experiments.

the principal colonic microbial metabolites of flavonoids, are also absorbed by MCT (*45*, *46*). The biological activities of these compounds should be studied extensively, particularly with respect to those that are poorly absorbed (*14*−*18*). We call them "metabo-nutrients" to distinguish them from classical nutrients and have addressed the physiological significance of MCT-mediated absorption of "metabo-nutrients" in terms of their health benefits (*46*).

In conclusion, we have demonstrated that CA has a higher efficiency of intestinal absorption than RA *in vivo*. When these results are taken together with the results of our previous *in vivo* study conducted under the same experimental conditions, the absorption efficiency of phenolic acids *in vivo* increases in the order: gallic acid = RA < CA < *p*-coumaric acid. This finding correlates well with the results obtained by *in vitro* studies using a Caco-2 cell system. The biological activities of flavonoids have been the center of much research interest; 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 the regular consumption of foodstuffs such as cereals and beer, a greater understanding of their pharmacological activity in relation to their absorption and distribution by MCT is highly desirable.

ABBREVIATIONS USED

MCT, monocarboxylic acid transporter; CA, caffeic acid; RA, rosmarinic acid; ECD, electrochemical detector.

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