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Relative Contribution of Small and Large Intestine to Deglycosylation and Absorption of Flavonoids from *Chrysanthemun morifolium* Extract

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The flower of *Chrysanthemum morifolium* Ramat (CM) is an established part of traditional Chinese medicine (TCM). Luteolin and apigenin flavonoids are the effective components of the CM extract (CME); however, they exist in the orally consumed CME as glycosides. The present study was carried out to determine the relative contribution of the small and large intestine to the deglycosylation and absorption of flavonoids from CME using a rat model system. The distribution of luteolin and apigenin in rat gastrointestinal (GI) luminal contents, tissues, and plasmas was assessed after the oral administration of CME. The hydrolysis and absorption of CME flavonoids in different rat GI segments were further evaluated by using in situ ligated models and cell-free extracts prepared from rat GI segments. The results demonstrated that after the oral administration of CME, the magnitude of deglycosylation in rats was surprisingly high (about 30%) in the stomach and upper intestine within the first 5 min after ingestion, and early absorption in the plasma was detected. The results from site-limited administration revealed that the stomach was the initial hydrolysis site, while the duodenum was the first effective absorption site for CME flavonoids. Diminishing microbial flora in the jejunum had no significant effect on the hydrolysis of the flavonoids from CME, but the cell-free extracts prepared from rat GI segments demonstrated a strong ability to hydrolyze. Taken together, our findings suggest that enteric disposition contributes to the pharmacokinetics of luteolin and apigenin after oral administration of CME. Moreover, the upper digestive tract plays a key role in the hydrolysis and absorption of flavonoids in CME.

KEYWORDS: *Chrysanthemum morifolium* extract; flavonoids; deglycosylation; absorption; luteolin; apigenin

INTRODUCTION

The flower of *Chrysanthemun morifolium* (CM), a widely used traditional chinese medicine (TCM) component, has a variety of health benefits, such as antioxidant activity (*1*), cardiovascular protective effects (*2*), hepato-protective action (*3*), anti-inflammatory and immunomodulatory activities (*4*). It has been demonstrated that luteolin and apigenin are the two main active ingredients in the flower of CM extract (CME) (*5*). Naturally occurring luteolin and apigenin in CME exist in their glycosylated forms luteolin-7-*O*-β-D-glucoside and apigenin-7-*O*-β-D-glucoside (*6*). Previous pharmacokinetic studies in our laboratory of luteolin and apigenin in CME (*7*, *8*) have demonstrated that the absorbed flavonoids are present in the systemic blood circulation in the forms of glucuronide, sulfate, and methylate conjugates, suggesting that glycosylated luteolin and apigenin undergo hydrolysis in vivo and release the bioactive aglycone for absorption or further reconjugation.

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The enteric disposition of the dietary flavonoid glycosides has proven to be an elusive and controversial issue over the years. It has traditionally been assumed that most of the flavonoid glycosides could not be absorbed from the small intestine and that cleavage of the β-glycosidic bond will not occur until the compounds reach the microflora of the large intestine (*9*). For instance, metabolites of rutin appear in blood circulation at 4 h after administration, indicating that the site of hydrolysis and absorption of rutin is the large intestine (*10*). However, several recent studies suggest the involvement of intestinal glycosidases in hydrolyzing certain monoglucosides, independently of their metabolism by the colonic microflora (*11*, *12*). Some flavones, such as apigenin, are hydrophobic and can be transported across membranes by passive diffusion in the small intestine (*13*, *14*), but the extent of absorption of dietary polyphenols in the small intestine is relatively small (10−20%) because of the high level of conjugation (*9*, *13*, *14*). Colonic absorption of flavonoids has been found in a single-pass perfused intestine segment model (*14*), but it is believed that there is no efficient absorption through the colonic tissue in rats (*15*). Briefly, the absorption and metabolism of dietary flavonoids are more complicated than previously thought.

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It is important to determine in which segment and to what extent flavonoids are hydrolyzed and absorbed so that better dosing regimens and formulations can be developed. Thus far, there have been no reports on integrating all of the intestinal disposition pathways for flavonoids in CME. Therefore, the present study was designed to systemically characterize the enteric disposition of luteolin and apigenin after the oral administration of CME by combining several in vivo and in vitro models. The results will undoubtedly contribute to the better understanding of the disposition and bioavailability of active flavonoids in CME.

MATERIALS AND METHODS

Chemicals. CME was provided by Institute of Medicine, Zhejiang University, China, and contained 7.60% of luteolin and 5.19% of apigenin, as determined by HPLC after being hydrolyzed with hydrochloric acid (*8*). Luteolin was purchased from J&K Chemical Co. Ltd. (Beijing, China). Apigenin was purchased from Sigma Co. Ltd. (St. Louis, MO, USA). Neomycin and tetracycline were purchased from Shenggong Biotech Co. (Shanghai, China). HPLC-grade methanol was purchased from Merck Co. Ltd. (Whitehouse Station, NJ, USA). All other chemicals were of analytical grade.

Animals. Male Sprague−Dawley rats weighing 180−200 g were obtained from Experimental Animal Center of the Zhejiang Academy of Medical Sciences. All procedures were approved by the Institutional Animal Care and Use Committee of Zhejiang University. The rats were housed in cages at a temperature between 20 and 23 °C, in a 12 h:12 h light−dark cycle, with food and water ad libitum. Animals were fasted for 12 h before experiments.

Absorption and Distribution of Luteolin and Apigenin in the Rat GI Tract. To determine the enteric distribution of luteolin and apigenin, CME was orally administered to each rat (200 mg/kg in 0.5% CMC-Na aqueous solution; $n=5$ per time point). At each time point (0.08, 0.5, 2, 4, 8, and 12 h after rats were gavaged), blood samples were collected from the tail vein into a heparinized tube, after which animals were anesthetized with intraperitoneal injection of 3% sodium pentobarbital (40 mg/kg). After the removal of the mesentery, the entire gastrointestinal tract was removed and sectioned as follows: (1) stomach; (2) duodenum (∼0.5−10.5 cm distal to the pyloris); (3) upper jejunum (∼11−31 cm distal to the pyloris); (4) terminal ileum (∼2−22 cm proximal to the ileocecal junction); (5) cecum; and (6) colon (segment after the cecum). Samples were collected according to the literature with minor modifications (*16*, *17*). Briefly, plasma was quickly separated by centrifugation (4000 rpm, 10 min). The GI contents of different segments were harvested and diluted to 5.0 mL with 0.9% NaCl saline. Tissues were cut into pieces and repeatedly washed with 0.9% NaCl saline after the removal of the mesentery and contents. The tissues were homogenized with 0.9% NaCl saline to make a 20% homogenate. The plasma samples were analyzed according to the method developed previously in our laboratory (*7*, *8*). The GI luminal contents and tissues were processed and analyzed as the plasma was, excluding the hydrolysis step. The assays for the analysis of luteolin and apigenin in GI contents and tissues were validated according to the guidance of the FDA for industry bioanalytical method validation. The concentration of luteolin and apigenin in GI contents and tissues was linear over the range of 0.25−100 μmol/L, the recoveries for both compounds at low, medium, and high concentration were in the range of 97.5−110.1%, and the intra- and interday precision of assays (expressed as RSD %) were below 8.1%.

Absorption and Distribution of Luteolin and Apigenin in Ligated Segments of the Rat GI Tract. Rats were anaesthetized with ip injection of sodium phenobarbital (40 mg/kg). The abdominal cavity was opened by a midline incision of 3−4 cm; a cannula was inserted into each of the GI segments, and each tied tightly with surgical silk to facilitate the site-limited administration of CME (5.0 mg/mL in 0.5% CMC−Na aqueous solution). The intestinal segment was then placed back into the abdominal cavity. The incision was sutured and covered by saline-wetted cotton, to keep the intestinal segment moist, and exposed to a heating lamp to maintain normal body temperature (*18*). The samples were collected and analyzed as described above at designated times.

To determine the regional hydrolysis and absorption potential of stomach and different intestinal segments, 30 male rats were allocated into six groups with five rats in each. Each group was employed to study the effect of stomach and different GI segments. Plasmas, luminal contents, and GI tissues were collected at 10 min after CME administration.

The jejunum was selected to conduct a site-limited pharmacokinetic study. Seven groups with five male rats each were employed, and blood samples were collected from one group at predosing (0 h) and at 0.08, 0.17, 0.5, 1.0, 1.5, and 2 h from the tail vein after jejunum-limited dosing; luminal contents and jejunum tissues were also collected from the other six groups at 0.08, 0.17, 0.5, 1.0, 1.5, and 2 h after jejunum-limited dosing.

To study the effect of flora located in the jejunum on hydrolysis and absorption of CME flavonoids, 15 male rats were allocated into three groups with five rats each: antibiotic-treated group, luminal content-free group, and saline-treated group. For the antibiotic-treated group, rats were preadministered antibiotics (400 mg/kg of neomycin and 200 mg/kg of tetracycline/day) in aqueous suspension by oral intubation for six consecutive days in order to suppress intestinal flora (*19*, *20*). For the luminal content-free group, rat intestinal tracts were emptied with isotonic sodium chloride at 0.2 mL/min with a peristaltic pump prior to CME administration, in order to establish a sterile ligated jejunum. The saline-treated group was used as the control group. The samples of plasma, jejunum luminal contents (for the luminal content-free group, gut residues were collected) and jejunum tissues were collected at 10 min after 10 mg of CME was administered to the jejunum.

Deglycosylation of CME Flavonoids in Cell-Free Extracts Prepared from Rat GI Segments. Rats were sacrificed by decapitation, and the GI tissues were removed and rinsed with ice-cold phosphate buffered saline (PBS; pH 7.0, 10 mmol/L). Samples were immediately cut into portions and homogenized in 4-fold ice-cold PBS (pH 7.0, 10 mmol/L), followed by centrifugation at 13,600*g* at 4 °C for 30 min. The resultant pellets were suspended in 250 mmol/L sucrose and stored at −80 °C until use (*12*). The protein content was measured by the Lowry method (*21*). CME was dissolved in dimethyl sulfoxide (DMSO) and then mixed with cell-free extract in PBS (pH 7.0, 10 mmol/L) with a final concentration of 0.5 mg/mL of protein and 37.5 μg/mL of CME; the DMSO was limited under 0.2% (v/v). The mixed samples were incubated at 37 °C for 30 min; the reaction was stopped by the addition of 10 μL of $HClO_4$ (30%) and then 0.5 mL of methanol, followed by centrifugation at 13,000 rpm for 5 min. A total of 50 μL of supernatant was analyzed by HPLC (*7*, *8*). Meanwhile, the heat-inactivated (100 °C, 5 min) cell-free tissue extract was also used as a negative control.

Data Analysis. Data are expressed as the means and standard deviations. C_{max} and T_{max} values were obtained directly from the observed concentration versus time data. Other parameters were estimated by noncompartmental methods using Winnolin5.2.

RESULTS

Gastrointestinal Distribution of Luteolin and Apigenin in Rats after Oral Administration of CME. Figures 1 and **2** show the temporal profiles of luteolin and apigenin in the GI contents and tissues from rats after oral administration of CME. The peak concentrations of luteolin and apigenin in the contents of the stomach (26.4% and 38.5%, respectively), duodenum (2.1% and 2.9%), and jejunum (4.3% and 6.2%) were found at 5−30 min after administration. The percent of original doses of the two compounds in the contents of ileum, cecum, and colon peaked from 2 to 8 h after administration, with peak values of 14.0% to 41.2%, respectively. As shown in **Figure 1**, the transit through the small intestine is rapid, on the basis of the high level of luteolin and apigenin present in the cecum contents at 2 h after administration. The total residence time of luteolin and apigenin in the GI tract exceeded 12 h (**Figure 1**).

The peak concentrations of luteolin and apigenin in the tissues of the stomach (69.9 and 46.3 nmol/g, respectively), duodenum (27.2 and 15.7 nmol/g), and jejunum (34.9 and 29.3 nmol/g) were also found at 5 min after administration, in parallel to the trend observed in the corresponding luminal contents. The peak values of luteolin and apigenin in the tissues of the large intestine were relatively low (2.5−11.4 nmol/g). Luteolin and apigenin were

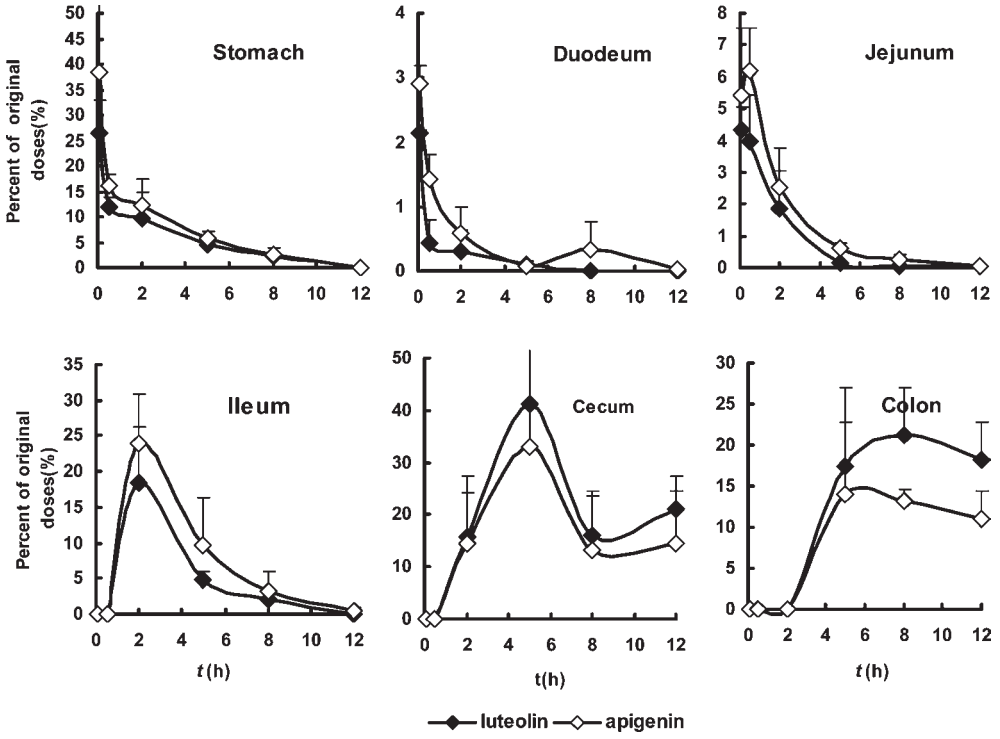

Figure 1. Distribution of luteolin and apigenin in the GI luminal contents at different times after oral administration of CME (200 mg/kg) to rats. Data are expressed as the mean ± SD, $n = 5$.

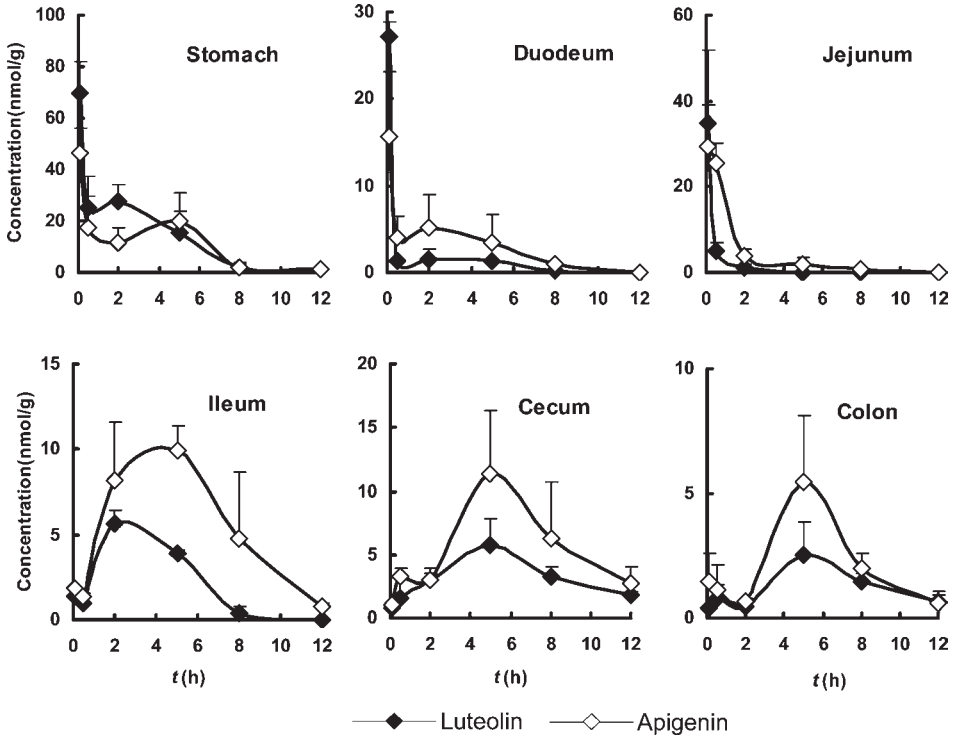

Figure 2. Distribution of luteolin and apigenin in GI tissues at different times after oral administration of CME (200 mg/kg) to rats. Data are expressed as the mean ± SD, $n = 5$.

distributed throughout all of the GI tissues examined at all time points. Even at the earliest time points, luteolin and apigenin were detectable in the colon tissue, while they were not detectable in the colon contents, suggesting that luteolin and apigenin were also able to distribute to the GI tract via systemic circulation (**Figure 2**).

Identification of the Site for Hydrolysis and Absorption of Flavonoids in CME in Rats. The hydrolysis and absorption site of flavonoids in CME were first evaluated in rats using an in situ site-limited administration model (**Figure 3**). The percent of original doses of luteolin and apigenin in the contents of all GI segments ranged from 20.6% to 74.5% of the original dose at 10 min, respectively. More aglycones were found in the large intestine than were found in the upper digestive tract. The percent of original doses of luteolin and apigenin in the cecum contents

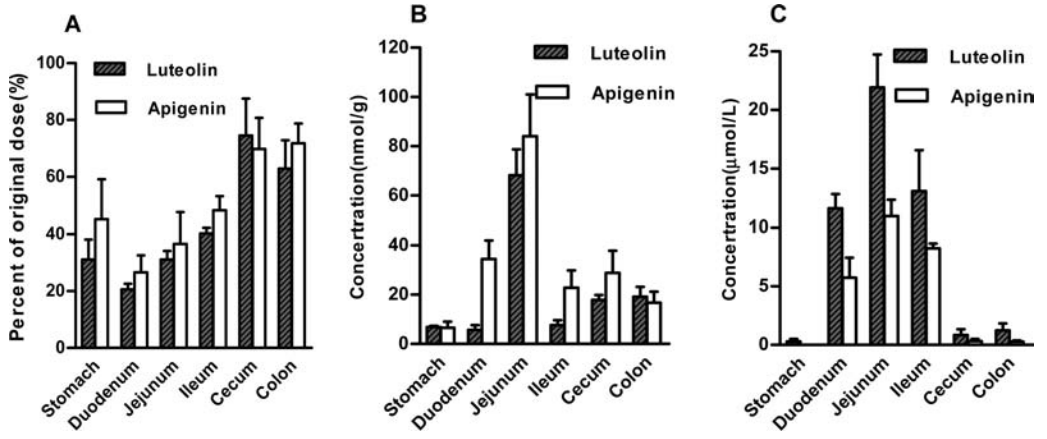

Figure 3. Amount of luteolin and apigenin in different GI luminal contents (**A**) and concentration in GI tissues (**B**) and plasma (**C**) at 10 min after the administration of 10 mg of CME. Data are expressed as the mean ± SD, $n = 5$.

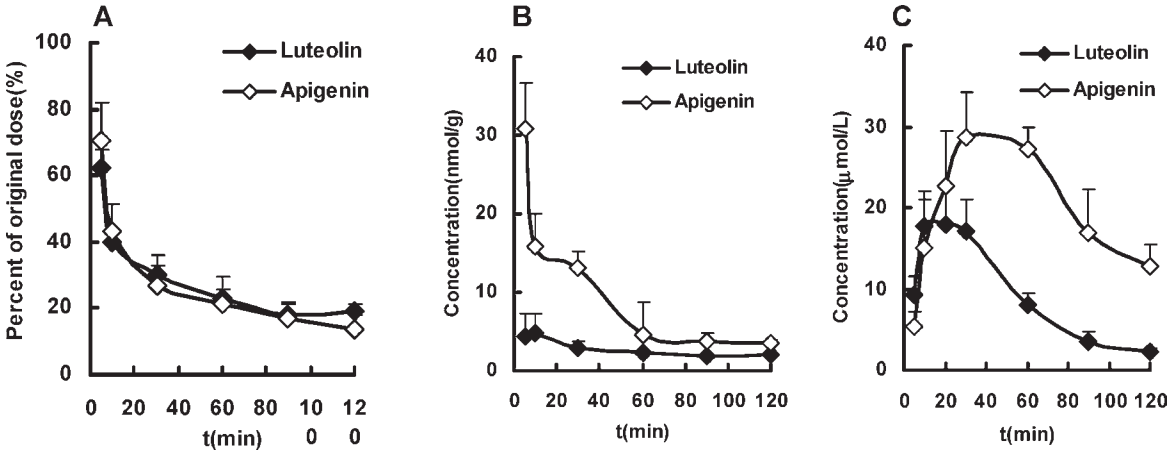

Figure 4. Time profiles of luteolin and apigenin in the jejunum contents (A), tissue (B) and plasma (C) at different times after in situ administration of 10 mg CME to rat jejunum (~11 to 31 cm distal to the pyloris). Data are expressed as mean ± SD, $n = 5$.

reached 74.5% and 69.8% of the original dose administered and 62.9% and 71.7% of the dose administered in the colon contents at 10 min after administration (**Figure 3A**).

Tissues from all of the segments were found to contain both luteolin and apigenin. Luteolin and apigenin in the jejunum (68.2 and 84.0 nmol/g, respectively) were 3−10 times higher than those in other tissues (**Figure 3B**). According to the plasma concentration levels of luteolin and apigenin after CME administration to different GI segments (**Figure 3C**), it could be deduced that the first effective absorption site was the duodenum, while the whole small intestine (duodenum, jejunum, and ileum) served as the main absorption site for both luteolin and apigenin. Apigenin and luteolin were absorbed in the large intestine (cecum and colon) to some extent but were only minimally absorbed in the stomach, although all of the GI tissues contained both luteolin and apigenin (**Figure 3C**). These results demonstrated clearly that the absorption of luteolin and apigenin in the study of rats was occurring predominantly in the small intestine (**Figure 3**).

Pharmacokinetics of Luteolin and Apigenin after Jejunum-Limited Administration. In order to evaluate the kinetics and mechanism of absorption, one part of the small intestine was chosen for focused study; the jejunum was selected because of its larger exchange area in comparison to that of all other available experimental tissues. Both hydrolysis and subsequent absorption were observed to be very efficient in the jejunum. Luteolin and apigenin appeared in the jejunum contents at 5 min postadministration (62.3% and 70.7% of the original dose, respectively), and the amount decreased rapidly to about 20% of the original dose at 2 h after administration (**Figure 4A**).

Table 1. Pharmacokinetic Parameters of Luteolin and Apigenin after Jejunum-Limited Administration of CME (Mean ± SD, $n = 5$)

pharmacokinetic parameters	luteolin	apigenin
$t_{1/2}$ (min)	30.58 ± 3.58	63.61 ± 23.5
T_{max} (min)	23.33 ± 11.6	40.00 ± 17.3
C_{max} (μmol/L)	20.02 ± 4.63	29.51 ± 1.30
AUC_{0-t} (μmol/L·min)	1187 ± 175	2456 ± 165
AUC_{0-t}/Dose (min/L)	421.6 ± 62.2	1354 ± 91

The concentration of luteolin in the jejunum tissue remained at a low level, with no significant change within 120 min. The concentration of apigenin in jejunum tissue peaked at 5 min (30.8 μmol/L) after jejunum-limited administration and decreased rapidly until it plateaued at 60 min (4.7 μmol/L) (**Figure 4B**). Blood kinetic analysis indicated efficient absorption, with C_{max} for luteolin and apigenin being 20 and 29.5 μmol/L, respectively (**Figure 4C**). The other parameters are shown in **Table 1**.

Effect of Antibiotics and Excluding the Luminal Contents on the Hydrolysis and Absorption of Flavonoids in CME in the Jejunum. The effect of flora in the jejunum was determined to be limited on the basis of the following results. (1) The concentrations of luteolin and apigenin in GI contents, tissue, and plasma only decreased by 20−30% in antibiotic-treated rats, as compared with those in the control rats. (2) Excluding the luminal contents of the jejunum only resulted in an approximately 20−30% decrease in luteolin

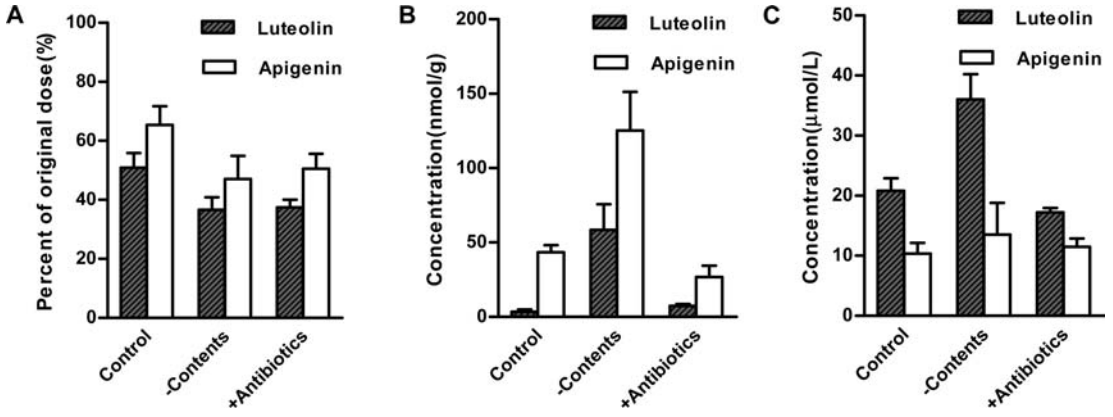

Figure 5. Amount of luteolin and apigenin in the jejunum contents (**A**) and concentration in jejunum tissue (**B**) and plasma (**C**) at 10 min after in situ administration (10 mg of CME) under various conditions. Data are expressed as the mean ± SD, $n = 5$.

and apigenin in GI contents (**Figure 5A**). (3) Antibiotics had no significant effect on the tissue and plasma concentrations of luteolin and apigenin after jejunum-limited administration, while excluding the luminal contents increased the tissue and plasma concentrations (**Figure 5B** and **C**).

Deglycosylation of CME Flavonoid Glycosides in Cell-Free Extracts Prepared from Rat GI Segments. Cell-free extracts prepared from rat GI segments were used to evaluate the role of intestinal hydrolyase and to investigate regional variations in the hydrolytic ability of GI tissue. CME flavonoids were rapidly deglycosylated by all investigated tissues to produce luteolin and apigenin. The hydrolytic potential of the jejunum was the highest, with the hydrolysis rate of luteolin/apigenin glycosides being 56.8% and 45.6%, respectively, of the amount administered at 10 min after administration, which was greater than rat cell-free extracts prepared from other regions. The hydrolytic ability of gastrointestinal tissues was ranked as follows: jejunum > duodenum ≈ ileum ≈ cecum ≈ colon > stomach (**Figure 6**).

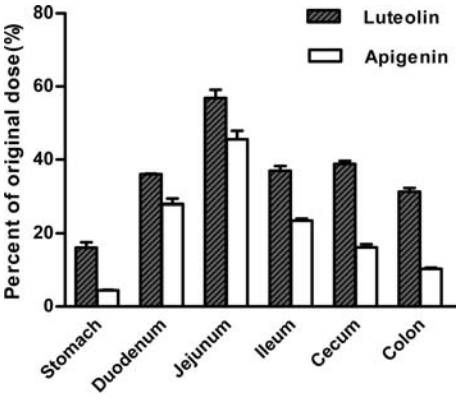

Figure 6. Deglycosylation rate of luteolin glucoside and apigenin glucoside in CME (37.5 μg/mL) after being incubated in cell-free extracts (0.5 mg protein/mL) prepared from rat GI segments at 37 °C for 30 min. Data are expressed as the mean ± SD, $n = 5$.

DISCUSSION

In our continued effort to characterize the bioavailability of CME flavonoids, we aimed in this study to determine how intestinal disposition affects bioavailability. For the first time, the GI segments were partitioned to identify to what extent CME flavonoids were hydrolyzed and absorbed by using a combination of several established in vivo and in vitro models. This investigation not only will contribute to the overall knowledge about the mechanism(s) of disposition and bioavailability of active flavonoids in CME but also will provide insights into other flavonoids.

The hydrolysis of glycosides to release the aglycones is a critical first step in their disposition because it serves as the initiator of all subsequent disposition processes (*11*, *12*). The fate of flavonoids in the stomach is important, which determines the forms of the compounds presented to the small intestine (*22*). Few studies exist on the ability of the stomach to alter the flavonoid structure. In the present study, though, the magnitude of deglycosylation of dietary flavonoid monoglucosides was surprisingly fast and high in the stomach contents (**Figures 1** and **3**), although the stomach did not show high ability to absorb in response to stomach-limited administration. The amount in the stomach content remained high for up to 2 h, suggesting that the stomach is a site for early hydrolysis and retention of the compounds. About 30% of the original doses were found after 10 min in the duodenum and jejunum contents after site-limited administration. Together with the absorbed amount, the total amount of compound hydrolyzed in jejunum would also be expected to be much higher. In fact, the amount of luteolin and apigenin in the cecum or colon contents was about 70% of the original dose in site-limited administration, suggesting the high hydrolytic potential of the large intestine (**Figure 3**). As CME flavonoids pass through the upper segment first and since the normal small intestinal length of the rat is approximately 80 cm, most of the hydrolysis must occur in the upper GI tract, diminishing the role of the large intestine.

Although the ability of the flora to hydrolyze CME flavonoids has been demonstrated in previous studies (*23*, *24*), our data clearly showed that intestinal glycosidases play a more prominent role than previously thought in the intestinal disposition of flavone glycosides. This conclusion is supported by the following results: (1) the exclusion of the luminal contents and use of antibiotics did not significantly reduce the formation of aglycones (**Figure 5**) and (2) the flavonoid glycosides were able to be metabolized in all of the cell-free extracts (**Figure 6**). This result was also partly indicated in the report by Hanske et al. wherein apigenin, luteolin, and their conjugates were detected in the urine and feces after the administration of apigenin glycosides to germ-free rats (*24*). Therefore, the importance of the mucosa of the upper segments on the hydrolysis of luteolin and apigenin glycosides should be increasingly recognized.

The absorption of luteolin/apigenin glysosides was also found to be region-dependent (**Figure 3**) (*9*, *13*, *14*) . The jejunum plays a more important role in absorption, while the role of the colon was limited. This is reasonable because absorption occurs less readily in the colon than in the small intestine due to the smaller exchange area and the lower density of transport systems (*25*). This result is further supported by the fact that (1) a maximum luteolin and

apigenin plasma concentration was reached after 30 min (*7*), which paralleled the maximal peak in GI content and tissue in the jejunum (**Figure 1** and **2**); (2) in the in situ pharmacokinetic assay, the amount of luteolin and apigenin in the jejunum contents was 62.3% and 70.7%, respectively, of the original at 5 min, and decreased to 39.7% and 43.3% of the original after 10 min, suggesting efficient absorption (**Figure 3**).

Compared with the result reported by Liu et al. (*14*), the absorption of luteolin and apigenin in the colon in the present site-limited experiment was not efficient. The reason may be that in the literature, the amount reduced was used to demonstrate drug absorption (*14*), while in the present study, the amount in the blood was used to directly demonstrate drug absorption. The differences might also be due to the fact that our model is used in the presence of normal flora, while the model in the literature was used in the absence of normal flora. In fact, excluding the luminal contents appeared to cause an increase in tissue uptake and plasma absorption (**Figure 5**).

Concentrations of luteolin and apigenin in duodenum tissue increased again and peaked at 2 h after administration. The reason may be that conjugated flavonoids are the substrates of efflux transporters (such as MRP2) and can be excreted to the bile and lumen. We further proposed that events of enterohepatic recycling may entail the reconversion of conjugates that are excreted by efflux transporters with subsequent hydrolysis by intestinal microflora and reuptake (*7*, *8*).

In the present jejunum-limited dosing experiment, the intestinal absorption of apigenin was more efficient than that of luteolin (**Figure 4C** and **Table 1**). AUC/dose (apigenin) versus AUC/dose (luteolin) was 3.2-fold. The percent of hydrolysis of luteolin in the jejunum in cell-free extracts was similar to that of apigenin (**Figure 6**), suggesting that the apigenin glycoside was not a better substrate of glycosidase. Thus, we deduced that more efficient absorption of apigenin results in higher AUC. The Papp value of apigenin was 2.3-fold that of luteolin in the Caco-2 cell line (*13*), which can at least partly support our proposal.

In our previous experiments, the intestinal absorption of apigenin was more efficient than that of luteolin after the administration of CME (**Figures 2** and **4**). AUC/dose (apigenin) versus AUC/dose (luteolin) was 16.0-fold when CME was orally administered to rats (*7*). This fold difference was higher than that found in the present site-limited administration study. Thus, other factors may be involved, such as intestinal transit time, enterohepatic circulation, and interactions among ingredients. With regard to the intestinal transit time, luteolin and apigenin were similar on the basis of their peak time in different segments (**Figures 1** and **2**); therefore, intestinal transit time was concluded to not act as the determining factor. Regarding the enterohepatic pathway, it was reported that luteolin and apigenin are able to undergo enterohepatic circulation in rats after oral dosing, where the drug excreted from bile is reabsorbed when it passes through the intestine to cause the concentration increase. Cumulative apigenin excreted in the bile was determined to be 6.34% of the initial dose administered, while that of luteolin was only 2.05% (*7*), which may be one reason for the better absorption of apigenin. Given the multi-ingredient character of herbal medicines, the likelihood of interactions among ingredients is increased. In previous experiments in our laboratory (*7*, *8*), AUC/dose (apigenin) versus AUC/dose (luteolin) was 16.0-fold when administrated in 200 mg/kg CME, while AUC/dose (apigenin) versus AUC/dose (luteolin) was 7.1-fold when administrated as pure luteolin and apigenin at approximately the same dose, suggesting that drug−drug interactions may affect the relative absorption potential of apigenin and luteolin.

On the basis of the present results, it can be concluded that enteric disposition contributes to the pharmacokinetics of luteolin and apigenin after oral administration of CME. Moreover, the upper digestive tract plays a key role in the hydrolysis and absorption of flavonoids in CME. The importance of upper segments on the hydrolysis and further absorption of luteolin and apigenin glycosides should be recognized, and efforts should be made to determine whether this location is also important for other compounds.

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