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Intestinal Absorption of Luteolin from Peanut Hull Extract Is More Efficient than That from Individual Pure Luteolin

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Luteoin is one of the main flavones and the crucial effective component of peanut hull extract (PHE). The present paper aims to elucidate the absorption mechanism of luteolin and clarify whether its absorption occurs primarily at a specific site of the intestine by an in situ single-pass intestinal perfusion (SPIP) model. Moreover, the paper investigates the difference in absorption of luteolin when it is administered in PHE form and as pure luteolin by the SPIP model and in vivo pharmacokinetics studies. Results showed that the effective permeability (P_{eff}) and absorption rate constant (k_a) of pure luteolin(5.0 μg/mL) in duodenum and jejunum were not significantly different, but markedly higher than that in the colon and ileum. The P_{eff} and k_a of luteolin in jejunum were concentration-independent, and the ATP inhibitor (DNP) did not influence P_{eff} and k_a of pure luteolin. However, the P_{eff} and k_a of luteolin in PHE were significantly greater than that of pure luteolin. The pharmacokinetics study showed that following oral administration of a single dose of pure luteolin (14.3 mg/kg) or PHE (= 14.3 mg/kg of luteolin) in rats, the peak concentration of luteolin in plasma (C_{max}) and the area under the concentration curve (AUC) for pure luteolin were 1.97 $\pm$ 0.15 μg/mL and 10.7 $\pm$ 2.2 μg/mL·h, respectively. These parameters were significantly lower than those of the PHE group ($P < 0.05$), C_{max} = 8.34 $\pm$ 0.98 μg/mL and AUC = 20.3 $\pm$ 1.3 μg/mL·h, respectively. It can be concluded that luteolin is absorbed passively in the intestine of rats and that its absorption is more efficient in the jejunum and duodenum than in the colon and ileum. The bioavailability of luteolin in PHE form is significantly greater than that of pure luteolin.

KEYWORDS: Luteolin; absorption mechanism; in situ single-pass intestinal perfusion model; peanut hull extract (PHE); pharmacokinetics

INTRODUCTION

Luteolin, a crucial member of the flavones, widely occurs in vegetables, fruits, and natural herbal drugs, such as Flos Chrysanthemi, Caulis Lonicerae japonicae, and Flos Lonicerae Japonicae, Aloe. Aside from its effects of vasodilation (*1*) and cancer prevention (*2*), recent studies have also shown that it could enter the cellular nuclei and suppress the oxidative damage of DNA (*3*). Our previous study also demonstrated that *Chrysanthemum morifolium* extract (CME) containing luteolin attenuated the reduction of contractile function and coronary flow of isolated rat heart caused by ischemia/reperfusion (*4*). Although the absorption of luteolin in Caco-2 cells and the intestine has been reported (*5, 6*), the absorption mechanism and difference in the extent of absorption in various intestinal segments have not been elucidated.

Peanut (*Arachis hypogaea* L.) is one of the most widely used nuts due to its nutrition and taste. Peanut hull extract (PHE) has been applied as a source of cosmetic antioxidant due to its potent antioxidative properties (*7, 8*). In China, peanut hull is a folk medicine for antihypertension and antihyperlipidemia, and a preparation containing the ethanol extract of peanut hull is recorded in the local pharmacopoeia of Yunnan Province for treatment of hyperlipidemia. Other preparations consisting of PHE have also been developed. It is believed that the pharmacological effects of PHE are mostly attributed to luteolin, one of the effective components in peanut hull. Although luteolin occurs in plants as the glycosylated form in most cases, in peanut hull, all of the luteolin is in the form of aglycone. PHE and luteolin extracted from peanut hull have been widely used in food additives and healthy foods. However, the bioavailability difference of luteolin being ingested in the form of PHE and pure luteolin is not clear. Because many other compounds co-occur with luteolin in PHE, it is thought that these concomitant components might influence the absorption of luteolin in vivo when ingested orally; however, this hypothesis has not been demonstrated.

In the present study, in situ single-pass intestinal perfusion (SPIP) has been applied to elucidate whether luteolin absorption

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occurs at a specific site of the intestine. The jejunum was selected to study the absorption mechanism of luteolin, and the permeabilities of luteolin in the form of pure luteolin and in PHE were compared. Finally, the oral bioavailabilities of luteolin in PHE form and as pure luteolin were compared in rats after administration of a single oral dose of 14.3 mg/kg of luteolin or 92.3 mg/kg PHE (= 14.3 mg/kg of luteolin).

MATERIALS AND METHODS

Chemicals. Luteolin (purity > 99%) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China; serial no. 111520-200201). PHE extracted from peanut hull with ethanol and purified with macroreticular resin, containing 15.3% of luteolin determined by HPLC, was provided by the Institute of Medicine, Zhejiang University, China. Methanol, HPLC grade, was purchased from Merck Co. Ltd. All other chemicals, including 2,4-dinitrophenol (DNP), were of analytical grade.

Animals. Male adult Sprague–Dawley rats (6−7 weeks of age, 200–250 g) were purchased from the Experimental Animal Center of Zhejiang Province, China. All of the animal work was performed under an approved animal use protocol of Zhejiang University. Animals were housed under standard conditions of light and dark cycles with free access to food and water, acclimated for at least 3 days, and fasted 12 h before the experiment.

In Situ Single-Pass Perfusion. The surgical procedure and the intestinal perfusion of a 10 cm isolated intestinal segment were performed as previously described (*9*). In brief, the rats were anesthetized with an intraperitoneal injection of 20% ethyl carbamate (1.0 g/kg) and placed on a warming pad under an infrared lamp to maintain body temperature at 37 °C. An inhalation anesthesia consisting of diethyl ether was used for the duration of the study. Upon verification of the loss of pain reflex, a 5-cm-long midline longitudinal incision was made, the intestinal segment of interest was located, and PVC tubing was cannulated. The outlet tubing was then cannulated about 10 cm aboral to the first opening. Both of the cannulae were secured with surgical silk sutures. The selected intestinal segment was then gently rinsed with prewarmed saline (37 ± 1 °C) and attached to the perfusion assembly. Care was taken to handle the small intestine gently and to minimize the surgery in order to maintain an intact blood supply. The entire surgical area was covered with a piece of sterilized absorbent gauze wetted with normal saline. Blank perfusion buffer was preinfused for 10 min by an infusion pump at a flow rate of 1.00 mL/min and followed by the perfusion buffer respectively containing luteolin (2.5, 5.0, and 10.0 μg/mL) or PHE (33.0 μg/mL, equivalent to 5.0 μg/mL of luteolin) and phenol red (20 μg/mL) as a nonabsorbable marker for measuring water flux at a flow rate of 0.20 mL/min, which were maintained at 37 ± 1 °C. Perfusate samples were collected into tared vials over 15 min intervals from the exit tubing for 2 h. All samples were stored at −20 °C until analysis. To prove the absorption was active or passive, the energy inhibitor, DNP (final concentration of 0.50 mM), was added to the perfusion buffer containing luteolin.

The perfusion solution was Krebs−Rings buffer consisting of NaCl (128.5 mM), KCl (4.7 mM), $MgCl_2$ (2.3 mM), $CaCl_2$ (3.3 mM), $NaH_2PO_4 \cdot 2H_2O$ (1.87 mM), $NaHCO_3$ (16.3 mM), and glucose (7.8 mM), which was adjusted to pH 6.8 with 1.0 M phosphoric acid. Luteolin or PHE and phenol red were dissolved in Krebs−Rings buffer to produce different concentrations of test solutions, respectively. Nonspecific absorption of tubing and syringe to drug could be neglected according to our test.

In Vivo Pharmacokinetics Study in Rats. Ten Sprague–Dawley male rats were randomly allocated into two groups of five rats each. After rats had fasted overnight with free access to water, each group was administered either PHE or luteolin (dissolved in 0.5% CMC−Na aqueous solution) by gavage at the dosage of 14.3 mg/kg of luteolin or 93 mg/kg of PHE (= 14.3 mg/kg of luteolin). Blood samples (0.2 mL/sample) were collected from the saphenous vein into tubes containing heparin at 0.17, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, and 24.0 h postdosing and predosing (0 h). The plasma was isolated immediately by centrifugation for 10 min at 4000 rpm and stored at −20 °C until analysis.

Sample Analysis. The samples were analyzed according to a method developed previously by our laboratory (*10*). Briefly, the plasma samples were hydrolyzed by acid and then were extracted by ethyl acetate. After the extraction was evaporated to dryness, the residue was reconstituted in mobile phase and analyzed by HPLC. The perfusate samples were diluted with methanol (1:1, v/v), the mixture was centrifuged at 13000 rpm for 10 min, and the supernatant was analyzed by HPLC. The HPLC analysis was performed on an Agilent SB-C_{18} (250 mm × 4.6 mm, 5 μm) column and a UV detector (Agilent G1314A); the mobile phase consisted of methanol, and 0.2% phosphoric acid aqueous solution (52:48, v/v) was isocratically pumped (Agilent G1310A isocratic pump) at 1.0 mL/min; the column temperature was maintained at 30 °C (Agilent G1316A temperature controller). According to the results of method validation, the method was suitable to determine luteolin in plasma or perfusate (data are not shown).

Data Analysis. The effective permeability (P_{eff}) was calculated on the basis of the inlet and outlet concentrations of luteolin (eq 1) (*11*)

$$P_{eff} = \frac{Q \ln(C_{in}/C_{out})'}{2\pi rL} \quad (1)$$

where Q is the flow rate of perfusion solution (0.20 mL/min), C_{in} and C_{out} are the inlet and outlet concentrations of luteolin in perfusion buffer, respectively, r is the radius of intestinal segment (0.25 cm), and L is the length of perfused segment measured after 120 min of perfusion. $(C_{in}/C_{out})'$ is the concentration ratio corrected for water flux. Correction for intestinal net water flux was done in outgoing drug concentration according to the method described by Sutton et al. (*12*). The intestinal absorption rate constant, k_a, was calculated for each 15 min interval, using eq 2 (*13*)

$$k_a = (1 - (C_{out}/C_{in})'\frac{Q}{V} \quad (2)$$

where V is the volume of perfused segment (=$r^2\pi$L).

The pharmacokinetic parameters, including peak plasma concentration (C_{max}), time to reach C_{max} (t_{max}), the area under concentration−time curve (AUC), and half-life of elimination ($t_{1/2}$), were calculated by DAS (V2.0) according to the concentration of luteolin in the plasma of rat after oral administration of luteolin and PHE.

RESULTS

Permeability of Luteolin in Different Intestinal Segments. The permeability of luteolin in different intestinal segments, including the duodenum, ileum, colon, and jejunum, was studied, and the steady state effective permeability (P_{eff}) and absorption rate constant (k_a) were calculated. The results showed that the P_{eff} and k_a of luteolin in the duodenum and jejunum were not significantly different, but markedly higher than those in the colon and ileum ($P < 0.05$) (see **Figure 1**).

Permeability of Luteolin in Jejunum Segment at Different Concentrations. Figure 2 shows the P_{eff} and k_a of luteolin in the jejunum segment from rats. Results revealed that both of these parameters were not concentration-dependent within the concentration range of 2.5–10 μg/mL.

Effect of DNP on the Absorption of Luteolin in the Jejunum. DNP is an inhibitor of ATP; results showed that DNP (0.50 mmol/L) did not influence P_{eff} and k_a of individual pure luteolin (5.0 μg/mL) in the jejunum (see **Figure 3**).

Difference in Absorption of Pure Luteolin Compared with That in PHE. Figure 4 shows the permeability parameters of luteolin from PHE and pure luteolin. Both the P_{eff} and k_a of luteolin in PHE were significantly higher than that of pure luteolin ($P < 0.05$). Hence, it could be deduced that the intestinal absorption of luteolin from PHE was prior to that of pure luteolin.

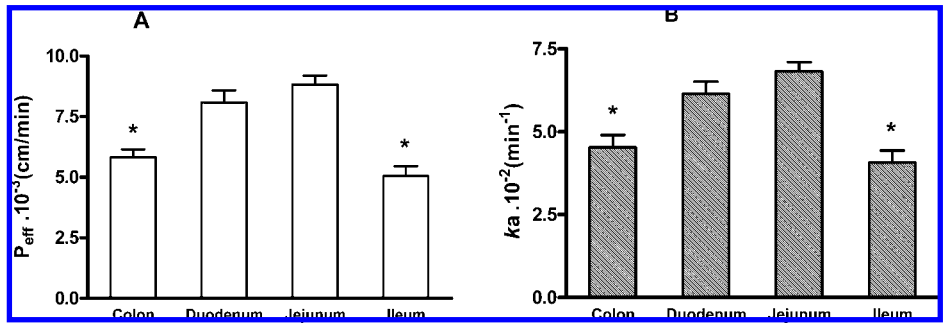

Figure 1. P_{eff} (**A**) and k_a (**B**) of luteolin (5.0 μg/mL) in different intestinal segments from rats. Data are expressed as mean ± SE ($n = 5$), compared with the jejunum or duodenum group; ∗, $P < 0.05$.

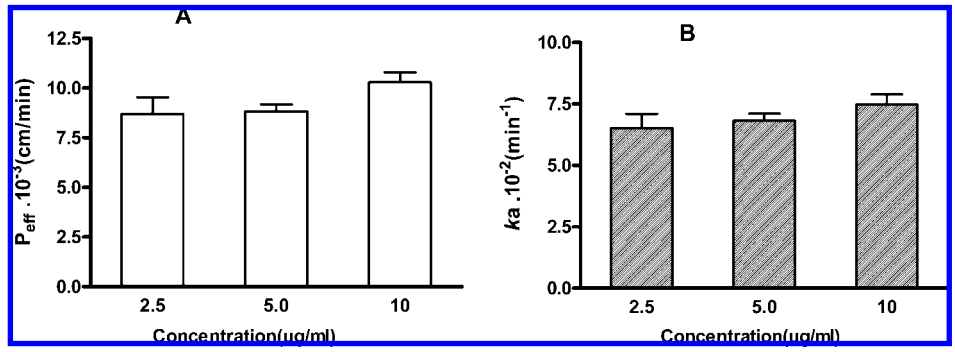

Figure 2. P_{eff} (**A**) and k_a (**B**) of luteolin in jejunum from rats within the concentration of 2.5−10 μg/mL. Data are expressed as mean ± SE ($n = 5$).

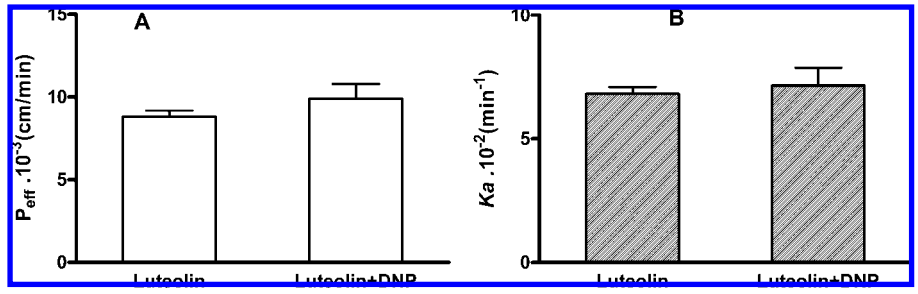

Figure 3. Effect of DNP on permeability parameters of luteolin (5.0 μg/mL) in the jejunum. Data are expressed as mean ± SE ($n = 5$).

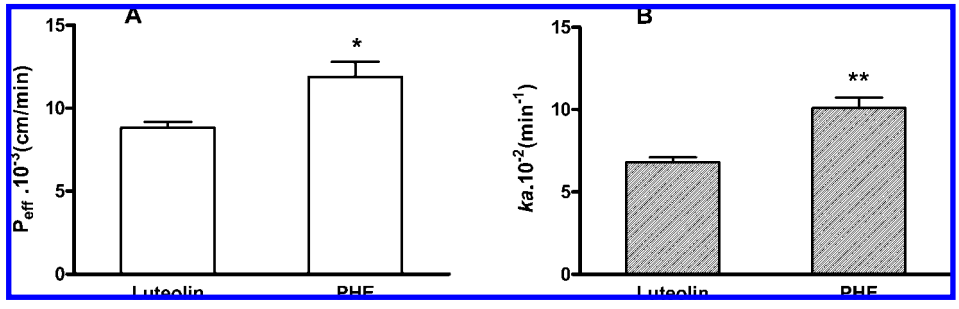

Figure 4. P_{eff} (**A**) and k_a (**B**) of luteolin in the jejunum from pure luteolin and PHE when the concentration of luteolin was 5.0 μg/mL. Data are expressed as mean ± SE ($n = 5$), compared with the luteolin group; ∗, $P < 0.05$.

Pharmacokinetics Difference of Pure Luteolin and PHE Luteolin. The concentration−time profile of luteolin in rat plasma after oral administration of luteolin (14.3 mg/kg) and peanut hull extract (92.3 mg/kg, = 14.3 mg/kg of luteolin) are shown in **Figure 5**. The concentration of luteolin increased sharply when rats received PHE, whereas the concentration of luteolin increased gradually when rats received pure luteolin. The pharmacokinetics parameters for luteolin are listed in **Table 1**. The $AUC_{0\sim\infty}$ and C_{max} in the PHE group were significantly higher than those of the luteolin group ($P < 0.01$), whereas the t_{max} of the PHE group was significantly lower than that of pure luteolin group ($P < 0.05$). The $t_{1/2}$ and CL in the PHE group were not markedly different from that of the pure luteolin group ($P > 0.05$).

DISCUSSION

From the in situ single-pass perfusion results, the P_{eff} of luteolin in the duodenum, ileum, colon, and jejunum was $>5.0 \times 10^{-3}$ cm/min, indicating that luteolin was highly absorbed almost in the whole intestine. Hence, it is a slightly soluble and highly permeable compound. Moreover, the P_{eff} in the

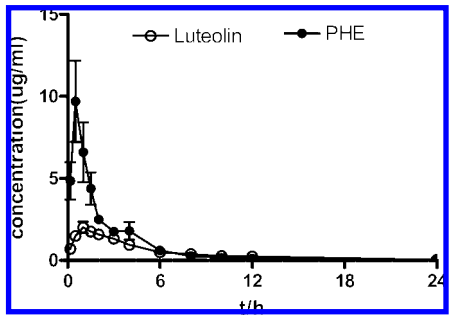

Figure 5. Mean plasma concentration−time profiles of luteolin in rats after oral administration of 14.3 mg/kg luteolin or 92.3 mg/kg PHE (= 14.3 mg/kg luteolin). Data are expressed as mean ± SE (n = 5).

Table 1. Pharmacokinetic Parameters of Luteolin after Oral Administration of Luteolin and PHE to Rats (Mean ± SE, n = 5)

parameter	luteolin	PHE[a]
$AUC_{0\sim\infty}$ (μg/mL·h)	10.7 ± 2.2	20.3 ± 1.3**
t_{max} (h)	1.02 ± 0.22	0.520 ± 0.05*
C_{max} (μg/mL)	1.97 ± 0.15	8.34 ± 0.98**
$t_{1/2}$ (h)	4.94 ± 1.2	2.772 ± 0.54
CL (L/h/kg)	1.36 ± 0.40	0.970 ± 0.09
V_d (L/kg)	4.27 ± 0.30	1.10 ± 0.12**

[a] *, $P < 0.05$; **, $P < 0.01$ compared with the luteolin group.

duodenum and jejunum was significantly higher than that in the colon and ileum segments, suggesting that luteolin was absorbed more efficiently in the duodenum and jejunum.

The jejunum was selected to study the mechanism of absorption, and results revealed that the P_{eff} of luteolin over the range of 2.5−10.0 μg/mL was not significantly different. Moreover, the inhibitor of ATP (DNP) did not influence the process of absorption, so it could be deduced that luteolin was absorbed passively in the intestine of rat.

PHE contains flavanones, especially eridictyol, which was reported to be metabolized to 7-eriodiotyol-glucuronide (*14*); moreover, its content in PHE is much lower than that of luteolin (*15*). Therefore, other flavonoids present in PHE could not be biotransformed into luteolin in the intestine after an oral administration of PHE. Otherwise, the peak of luteolin was confirmed by DAD detector and MS analysis, which revealed that concomitant components would not influence luteolin determination. Hence, from the results of intestinal perfusion as well as in vivo pharmacokinetic study, it could be concluded that luteolin in PHE was absorbed more efficiently than that of pure luteolin, suggesting that co-occurring components in the PHE might stimulate luteolin to be absorbed in the intestine. The same result was also observed in our previous study with *Chrysanthemum morifolium* extract (CME), in which rats were administered 200 mg/kg CME (= 14.3 mg/kg luteolin, which occurred in the form of luteolin-7-glucoside). The AUC and C_{max} in that study were 23.0 μg/mL·h and 3.87 μg/mL, respectively, and these parameters were also 2-fold those obtained with pure luteolin in the present study. This result also demonstrated that the co-occurring components in CME could enhance the absorption, even the luteolin present in CME in the form of glycoside. Generally, it is believed that the oral bioavailability of flavonoids is very low; however, according to our results, the bioavailability of luteolin in herbal or plant extracts was much higher than that of pure luteolin. In contrast, Zuo et al. (*16*) reported that the co-occurring components in hawthorn phenol extract had no significant effect on the intestinal absorption of the three major hawthorn flavonoids: hyperoside, isoquercitrin, and epicatechin.

A double-peak in the plasma concentration profile of luteolin and apigenin in rat was found in our pharmacokinetics study of CME; however, in the present study, the double-peak was not found in either of the study groups. Double-peaks are common in pharmacokinetics of flavonoids. Generally, it is assumed that the second peak is caused by an enterohepatic pathway, where the drug excreted from bile is reabsorbed when it passes through the intestine to cause the concentration increase a second time. Another reason is that the flavoloid aglycone is absorbed rapidly to form the first peak of concentration, and the flavonoid glucoside is absorbed after hydrolysis to cause the second peak. Actually, the luteolin was detected in bile when rats orally ingested CME (contain luteolin-7-glucoside and apigenin-7-glucoside), but the total excretion of luteolin within 36 h was just 2.05% of the total dose (*17*). Bile excretion of luteolin was also found after rats received pure luteolin; however, the second peak did not exist in the concentration−time profile. Therefore, the enterohepatic circulation played a limited role in the formation of the double-peak pharmacokinetics profile.

In conclusion, the results of the present study show that luteolin is absorbed passively by the intestine of rat and that the absorption is more efficient in the duodenum and jejunum than in the colon and ileum. The bioavailability of luteolin in PHE is significantly higher than that of pure luteolin.

ABBREVIATIONS USED

PHE, peanut hull extract; CME, *Chrysanthemum morifolium* extract; SPIP, in situ single-pass intestinal perfusion; DNP, 2,4-dinitrophenol; P_{eff}, effective permeability; k_a, absorption rate constant; C_{max}, peak concentrations; AUC, area under the concentration versus time curve; $t_{1/2}$, elimination half-life; CL, plasma clearance; V_d, apparent volume of distribution.

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