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Food−Drug Interaction between Ferulic Acid and Nateglinide Involving the Fluorescein/H^+ Cotransport System

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In clinical, patients usually take many kinds of drugs at the same time. Thus, drug−drug interactions involving transporters can often directly affect the therapeutic safety and efficacy of many drugs. However, there have been few studies on food−drug interactions involving transporters. Dietary polyphenols have been widely assumed to be beneficial to human health. Polyphenols are commercially prepared and used as functional foods. We report here for the first time that ferulic acid, which is widely used as a functional food, affects the transport of clinical agents. It is important to be aware of the potential of food−drug interactions and to act in order to prevent undesirable and harmful clinical consequences.

KEYWORDS: Nateglinide; fluorescein; ferulic acid; polyphenol; monocarboxylate transporter

INTRODUCTION

Dietary polyphenols are thought to be beneficial to human health by exerting various biological effects such as free-radical scavenging, metal chelation, modulation of enzymatic activity, and alteration of signal transduction pathways (*1*−*3*). Epidemiological studies have shown relationships between consumption of polyphenol-rich foods and prevention of diseases such as cancer, coronary heart disease, and osteoporosis, promoting interest in polyphenols (*4*−*6*). Polyphenols are classified into phenolic acids, flavonoids, and less common stilbenes and lignans. Many studies have focused on the absorption and metabolism of flavonoids (*7*−*9*). However, there have been few studies on phenolic acids, such as ferulic acid and caffeic acid (*10*−*11*), despite their high contents in fruits, cereals, and some vegetables (*12*). Recently, it has been reported that ferulic acid is transported across human intestinal Caco-2 cells by monocarboxylate transporter (MCT) (*13*), as is the case for fluorescein (*14*).

Nateglinide has been used as a new oral hypoglycemic agent (*15*). The pharmacokinetic features of nateglinide may be attributable to its rapid intestinal absorption. However, its physicochemical features are incompatible with rapid absorption by passive diffusion. Because nateglinide is an anionic compound with $pK_a = 3.1$, it exists predominantly in ionized form at the intestinal physiological pH of 6.5. Moreover, its chloroform/water partition coefficient has been reported to be 0.2 at pH 6.8, indicating that it is scarcely lipophilic (*16*). Because of these features, it had been suggested that nateglinide is absorbed via a specific transport system(s) in the intestine. Recently, we have reported that nateglinide shares a transporter with fluorescein (*17*).

It is well-known that drug−drug interactions involving transporters can often directly affect the therapeutic safety and efficacy of many drugs. However, there have been few studies on food−drug interactions involving transporters. Polyphenols, including ferulic acid, are commercially prepared and used as functional foods. It is possible that a daily diet of functional foods reduces the oral bioavailability of nateglinide. This study was designed to investigate the food−drug interactions involving the intestinal fluorescein/H^+ cotransport system.

MATERIALS AND METHODS

Chemicals. Ferulic acid was purchased from Sigma (St. Louis, MO). Nateglinide was kindly donated by Yamanouchi (Tokyo, Japan). Fluorescein was purchased from Nacalai Tesque (Kyoto, Japan). All other reagents were of the highest grade available and used without further purification.

Cell Culture. Caco-2 cells obtained from American Type Culture Collection (Rockville, MD) were maintained in plastic culture flasks (Falcon, Becton Dickinson and Co., Lincoln Park, NJ) as described previously (*18*). The medium used for growth of Caco-2 cells was Dulbecco's modified Eagle's medium (Gibco, Life Technologies, Inc., Grand Island, NY) with 10% fetal bovine serum (ICN Biomedicals, Inc., Aurora, OH), 1% nonessential amino acids (Gibco), 4 mM glutamine (Gibco), and 100 IU/mL penicillin−100 μg/mL streptomycin. The monolayer cultures were grown in an atmosphere of 5% CO_2/95% O_2 at 37 °C. Cells reached confluency after 4−6 days in culture. The cells were harvested with 0.25 mM trypsin and 0.2% EDTA (5 min at 37 °C), resuspended, and seeded into a new flask. Cells between the 44th and 52nd passages were used in this study. For uptake study, Caco-2 cells were seeded at a cell density of $(1-3) \times 10^5$ cells/cm^2 on

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12-well plastic plates (Corning Costar Corp., Cambridge, MA). The cell monolayers were fed a fresh growth medium every 2 days and were then used at 4−6 days for the uptake experiments.

Uptake Studies. The uptake experiment was performed as described previously (*19*). The uptake of substrates was measured using monolayer cultures grown in 12-well plastic plates. The composition of the incubation medium was as follows: 137 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.4 mM KH_2PO_4, 0.3 mM NaH_2PO_4, 4.2 mM $NaHCO_3$, 25 mM d-glucose, and 10 mM MES (pH 6.0) or HEPES (pH 7.4). After removal of the growth medium, cells were preincubated at 37 °C for 10 min with 1 mL of incubation medium. After removal of the medium, 0.5 mL of incubation medium containing substrates was added. The monolayers were incubated for a designed time at 37 °C. Each cell monolayer was washed rapidly twice with 1.0 mL of an ice-cold incubation medium at the end of the incubation period. The cells were suspended in 0.4 mL of an extraction solution (1 N H_3PO_4/methanol = 50:50) for 1 h at room temperature. The extraction solution was used for the determination of the substrate concentration after centrifugation at 12000*g* for 10 min.

Analytical Procedures. Substrates were determined using an HPLC system equipped with a JASCO 880-PU pump 870-UV UV−vis detector. The column was a CERI L-column ODS (4.6 mm inside diameter × 150 mm). In the assay for ferulic acid, a mobile phase containing acetonitrile/50 mM acetate at pH 4.0 (15:85) was used. The column temperature and flow rate were 40 °C and 1.0 mL/min, respectively. Wavelength for detection of ferulic acid was 320 nm. In the assay for nateglinide, a mobile phase containing acetonitrile/50 mM H_3PO_4 at pH 2.5 (45:55) was used (*20*). Column temperature and flow rate were 55 °C and 0.7 mL/min, respectively. Wavelength for detection of nateglinide was 210 nm. The lower limits of quantitation for ferulic acid and nateglinide were 500 and 250 pmol/mL, respectively. The reproducibility was good without requiring any sample pretreatment. The retention time for ferulic acid and nateglinide are 10.0 and 8.5 min, respectively. Protein was measured by the method of Lowry et al. with bovine serum albumin as a standard (*21*). Statistical significance was evaluated using one-way analysis of variance (one-way ANOVA) or unpaired Student's *t* test. A value of $p < 0.05$ was considered significant. Nonlinear regression analysis and least-squares fitting for the Eadie−Hofstee plot of substrate uptake were performed by using Origin (version 6.1J).

RESULTS

Uptake of Ferulic Acid by Caco-2 Cells. It has been reported that ferulic acid transport was dependent on pH and in a vectorical way in the apical-to-basal direction (*13*). However, Konishi et al. reported that acetic acid inhibited the transcellular transport of fluorescein by Caco-2 cells, but Kuwayama et al. reported no inhibition by acetic acid of the uptake of fluorescein by Caco-2 cells (*14*, *22*). It is possible that differences between the transcellular transport and uptake experiments are due to other MCTs in the basolateral membrane. In this study, we examined the uptake of ferulic acid and nateglinide by Caco-2 cells to investigate the food−drug interactions involving the apical-localized fluorescein/H^+ cotransport system. Because the Caco-2 cells were grown in wells, basolateral transporters make a minor contribution to the transport of substrates (*23*).

To determine whether the H^+-driven transport system is involved in the uptake of ferulic acid by Caco-2 cells, the uptake of ferulic acid was examined in the presence and absence of FCCP (a protonophore). The uptake of ferulic acid was markedly reduced by the addition of FCCP (**Figure 1**).

The concentration dependence of the uptake of ferulic acid was examined. In the absence of an H^+ gradient, the uptake of ferulic acid was not saturated up to 12.5 mM (**Figure 2**). Transporter-mediated uptake of ferulic acid was saturated at a higher ferulic acid concentration. The K_m and V_{max} values were determined by kinetic analysis to be 2.50 mM and 55.0 nmol (mg of protein)$^{-1}$ (20 s)$^{-1}$, respectively.

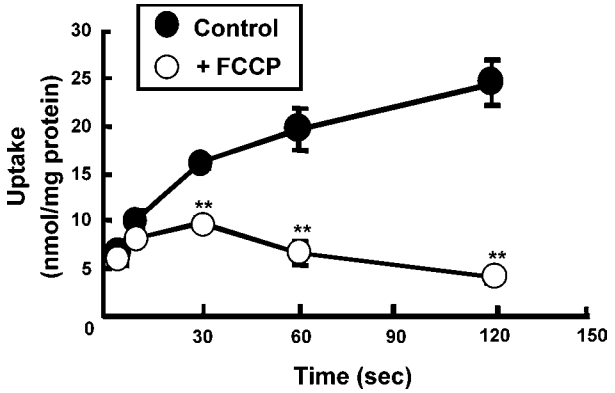

Figure 1. Effect of FCCP on the uptake of ferulic acid by Caco-2 cells. The uptake of 500 μM ferulic acid by Caco-2 cells was measured at pH 6.0 in the presence or absence of 50 μM FCCP. Each point represents the mean with SD of 3–4 determinations. $^{**}p < 0.01$, significantly different from that in the absence of FCCP.

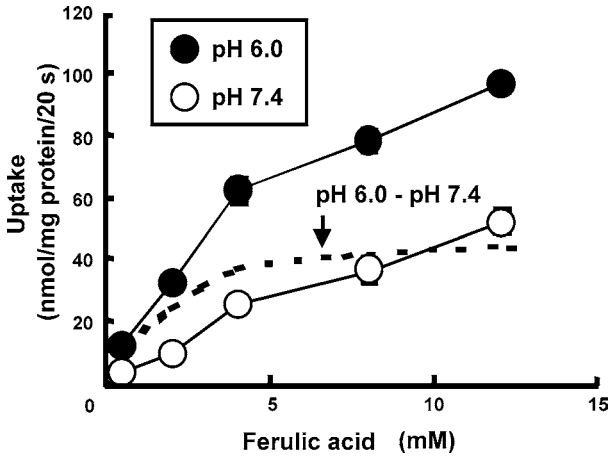

Figure 2. Concentration dependence of the uptake of ferulic acid by Caco-2 cells. Uptake of ferulic acid was measured for 20 s. The dashed line represents transporter-mediated uptake obtained as the difference between the uptake in the presence of the H^+ gradient and that in the absence of the H^+ gradient. Each point represents the mean with SD of 4–10 determinations.

We investigated the effects of fluorescein and nateglinide on the uptake of ferulic acid. As shown in **Figure 3**, both fluorescein and nateglinide significantly reduced the uptake of ferulic acid.

Uptake of Nateglinide by Caco-2 Cells. To clarify whether nateglinide shares a transporter with ferulic acid, the opposite inhibitory effect of ferulic acid on the uptake of nateglinide was investigated. As shown in **Figure 4**, ferulic acid significantly reduced the uptake of nateglinide.

We then investigated the kinetics of the inhibitory effect of ferulic acid on H^+-driven nateglinide uptake. **Figure 5** shows the effect of ferulic acid on the uptake of nateglinide in terms of a Lineweaver−Burk plot. Ferulic acid inhibits the uptake of nateglinide in a competitive manner (K_i = 1.20 mM).

DISCUSSION

Hydroxycinnamic acids are a group of polyphenols that are widely distributed in the diet, mostly in whole grains, fruits, vegetables, and beverages (*12*, *24*−*25*). Polyphenols are attracting increasing interest because of their strong antioxidant activity, which has been associated with beneficial effects of polyphenol-rich diets on human health (*26*). Ferulic acid can

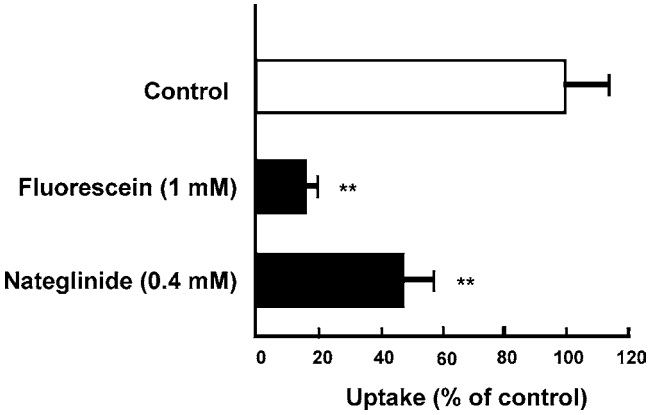

Figure 3. Inhibitory effects of fluorescein and nateglinide on H^+-driven ferulic acid (500 μM) uptake by Caco-2 cells. Uptake of ferulic acid was measured for 30 s. Each point represents the mean with SD of 4 determinations. The control value of the uptake of ferulic acid was 9.72 $\pm$ 0.82 nmol (mg of protein)$^{-1}$ (30 s)$^{-1}$. **p < 0.01, significantly different from the control.

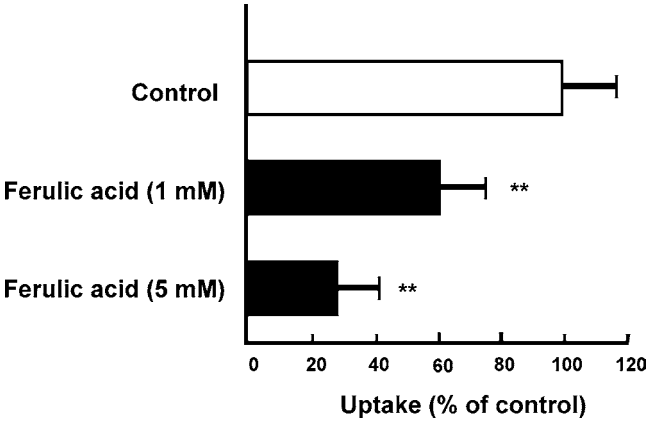

Figure 4. Inhibitory effect of ferulic acid on H^+-driven nateglinide uptake by Caco-2 cells. The uptake of nateglinide (50 μM) by Caco-2 cells was determined in the presence or absence of ferulic acid. Uptake of nateglinide was measured for 5 min. Each column represents the mean with SD of 4 determinations. The control value of the uptake of nateglinide was 10.9 $\pm$ 1.94 nmol (mg of protein)$^{-1}$ (5 min)$^{-1}$. **p < 0.01, significantly different from the control.

readily form a resonance-stabilized phenoxyl radical, which accounts for its potent antioxidant activity. Because ferulic acid has been shown to be effective against cancers, cold, flu, skin aging, muscle wasting, and influenza, ferulic acid is now commercially prepared and used as a functional food.

Transporter-mediated drug−drug interactions involving drugs that have a narrow therapeutic range may have serious adverse consequences. However, there have been few studies on food−drug interactions involving transporters. Because it has been reported that ferulic acid interacts with fluorescein, we have focused on the intestinal fluorescein transporter (*14*). Moreover, we have recently reported that the intestinal fluorescein transporter contributes to the uptake of nateglinide, an oral hypoglycemic agent (*17*). These findings suggest that nateglinide interacts with not only coadministered drugs but also functional foods. It is important to evaluate food−drug interactions involving the intestinal fluorescein transport system. We report here for the first time that ferulic acid, which is widely used as functional food, affects the transport of clinical agent.

The uptake of ferulic acid from the apical membranes of Caco-2 cells increased with a decrease in extracellular pH (data not shown). In addition, FCCP significantly decreased the uptake of ferulic acid. These results indicate that the uptake of ferulic acid is associated with an H^+-driven transport system. Because the intestinal nateglinide/fluorescein uptake system is an H^+-dependent system (*17*, *20*), we examined the inhibitory effects of fluorescein and nateglinide on the uptake of ferulic acid. Both fluorescein and nateglinide inhibited the uptake of ferulic acid. These results suggest that ferulic acid transport occurs via an H^+-dependent system that is identical to the nateglinide/fluorescein transport system.

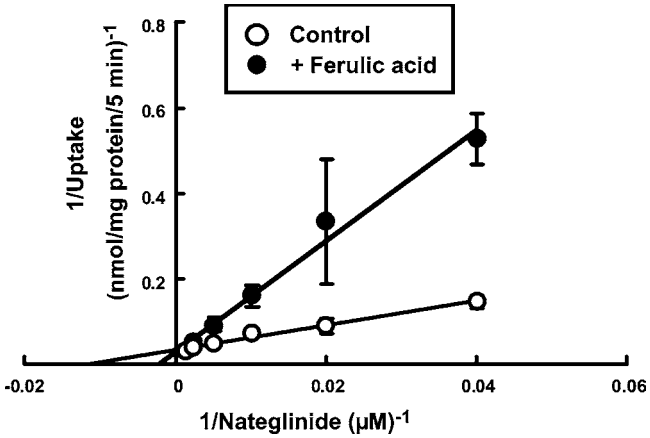

Figure 5. Lineweaver–Burk plot of H^+-driven nateglinide uptake by Caco-2 cells. Uptake of nateglinide was measured for 5 min in the presence or absence of 5 mM ferulic acid. Each point represents the mean with SD of 3–5 determinations.

In the present study, the opposite inhibitory effect of ferulic acid on nateglinide uptake was investigated. Ferulic acid competitively inhibited H^+-dependent nateglinide uptake. Furthermore, the estimated K_i value of ferulic acid for nateglinide uptake (1.20 mM) is similar to the K_m value for the saturable uptake of ferulic acid (2.50 mM). Moreover, the K_i value for nateglinide uptake was close to the K_i values for fluorescein transport (2.99 mM) and for benzoic acid transport (1.20 mM) (*14*). Our conclusion was further supported by these findings.

The absorption of drugs from the gastrointestinal tract is one of the important determinants for oral bioavailability. The recent development of molecular biological techniques has led to the identification of drug transporters responsible for the intestinal absorption of a wide variety of drugs (*27*). It is widely recognized that drug transporters contribute to the absorption of administered drugs from the intestine. Because patients usually take many kinds of drugs at the same time, it is possible that drug interactions at intestinal absorption level are caused by the inhibition of transporters in the intestine. Drug−drug interactions involving peptide transporter 1 (PEPT1) and MCTs are likely to occur because of their broad substrate specificities. Besides PEPT1 and MCTs, we have to be aware of drug−drug interactions involving the fluorescein/H^+ cotransport system.

In addition to drugs, several dietary constituents may be important factors affecting drug absorption and disposition. However, a pharmacist consults the patient before dispensing medicine, whereas a salesperson is not expected to do so before selling food. It is important to be aware of the potential of food−drug interactions and to act in order to prevent undesirable and harmful clinical consequences. It is possible that food−drug interactions involving the fluorescein/H^+ cotransport system reduce the oral bioavailability of drugs. In this study, we only focused on ferulic acid. Besides ferulic acid, we intake other polyphenols such as *p*-coumaric acid, caffeic acid, or chlorogenic acid in the daily diet. The total daily polyphenol intake

(in order of 1 g/day) is greater than the recommended daily dose of many kinds of drugs (in order of 10 or 100 mg/day) (*28*). To evaluate the inhibitory effect of total polyphenol on the fluorescein/H^+ cotransport system, it is important to elucidate the effects of every polyphenol on the fluorescein/H^+ cotransport system.

In summary, we report here for the first time that ferulic acid, which is widely used as functional food, affects the transport of the clinical agent. The results suggest that ferulic acid transport occurs via an H^+-dependent system that is identical to the nateglinide/fluorescein transport system. It is important to be aware of the potential of food−drug interactions and to act in order to prevent undesirable and harmful clinical consequences.

ABBREVIATIONS USED

MCT, monocarboxylate transporter; PEPT1, peptide transporter 1.

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