Inhibitory Effect of Caffeic Acid on Human Organic Anion Transporters hOAT1 and hOAT3: A Novel Candidate for Food–Drug Interaction

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Summary: Several kinds of food have been shown to influence the absorption and metabolism of drugs, although there is little information about their effect on the renal excretion of drugs. In this study, we performed uptake experiments using Xenopus laevis oocytes to assess the inhibitory effects of chlorogenic acid, caffeic acid and quinic acid, which are contained in coffee, fruits and vegetables, on human organic anion transporters hOAT1 and hOAT3; these transporters mediate renal tubular uptake of anionic drugs from blood. Injection of hOAT1 and hOAT3 cRNA into oocytes stimulated uptake of typical substrates of hOAT1 and hOAT3 (p-aminohippurate and estrone sulfate, respectively); among the three compounds tested, caffeic acid most strongly inhibited these transporters. The apparent 50% inhibitory concentrations of caffeic acid were estimated to be 16.6 µM for hOAT1 and 5.4 µM for hOAT3. Eadie-Hofstee plot analysis showed that caffeic acid inhibited both transporters in a competitive manner. In addition to the transport of p-aminohippurate and estrone sulfate, that of antifolates and antivirals was inhibited by caffeic acid. These findings show that caffeic acid has inhibitory potential against hOAT1 and hOAT3, suggesting that renal excretion of their substrates could be affected in patients consuming a diet including caffeic acid.

Keywords: hOAT1; hOAT3; caffeic acid; transport; food–drug interaction

Introduction

In the renal proximal tubule, organic anion transporters mediate the tubular secretion of various drugs, including antibiotics, antivirals, antitumor agents and diuretics, and contribute to the regulation of their blood concentrations. In particular, human organic anion transporters hOAT1 (SLC22A6) and hOAT3 (SLC22A8), expressed in the basolateral membrane of proximal epithelial cells, are thought to play the main roles in the tubular uptake of these compounds from blood. Accordingly, simultaneous administration of a drug that inhibits hOAT1 and hOAT3 and their substrate drugs would result in drug–drug interaction. For instance, it is well known that probenecid, a typical inhibitor of renal organic anion transporters, elevates blood levels of cephalosporins, and it is accepted that hOAT3 is involved in this drug–drug interaction. Among the drug–drug interactions that are mediated by renal organic anion transporters, the combination of antifolate methotrexate and nonsteroidal anti-inflammatory drugs (NSAIDs) is one of the most serious, because this interaction may cause severe toxicity or death when high-dose methotrexate is used to treat malignancies. This is considered to be attributable to the inhibition by NSAIDs of hOAT1- and hOAT3-mediated uptake of methotrexate.

On the other hand, probenecid is co-administered with the antiviral cidofovir to prevent the nephrotoxicity induced by hOAT1-mediated uptake of the antiviral. Therefore, inhibition of hOAT1 and hOAT3 is sometimes useful and sometimes unfavorable.

Since grapefruit juice was reported to increase the bioavailability of felodipine, attention has been paid to food–drug interactions. Subsequently, several types of food–drug interaction have been demonstrated, and their mechanisms have been elucidated at the molecular level. Although many ingredients of food and herbs have been shown to interact with drug transporters in the small intestine and with drug metabolic enzymes in the small intestine and liver, there is little information on food–drug interactions via hOAT1 and hOAT3. So far, only ellagic acid and flavonoids have been reported to interact with the transporters.
The purpose of the present study was to examine the inhibitory effects of chlorogenic acid, caffeic acid and quinic acid on hOAT1 and hOAT3 by performing uptake experiments using Xenopus laevis oocytes and to evaluate the clinical significance of these effects. Chlorogenic acid is contained in fruits and vegetables, but coffee is its major source in the human diet. Coffee has been shown to decrease the risk of diabetes, hepatocellular carcinoma and hypertension, and antioxidant chlorogenic acid is thought to contribute to these effects. As illustrated in Figure 1, chlorogenic acid is an ester formed between caffeic acid and quinic acid. Chlorogenic acid is absorbed as an intact molecule, and as caffeic acid and quinic acid after hydrolysis in the gastrointestinal tract. Caffeic acid is also reported to have biological effects, including antitumor, antimetastatic, antioxidant, anti-hyperglycemic and anti-inflammatory properties. The findings obtained from this study suggest the possibility of a novel food–drug interaction.

Materials and Methods

Materials: [3H]-Aminohippurate (4.53 Ci/mmol) and [3H]-estron sulfate (57.3 Ci/mmol) were obtained from PerkinElmer Life Science (Boston, MA, USA). [3H]-Amino-pterin (35.1 Ci/mmol), [3H]-methotrexate (24.0 Ci/mmol), [3H]-acyclovir (14.2 Ci/mmol) and [3H]-penciclovir (10.6 Ci/mmol) were purchased from Moravek Biochemicals (Brea, CA, USA). Caffeic acid was from Enzo Life Sciences International, Inc. (Plymouth Meeting, PA, USA). Chlorogenic acid and quinic acid were obtained from Cayman Chemical Company (Ann Arbor, MI, USA) and ChromaDex (Irvine, CA, USA), respectively. Unlabeled p-aminobipropionate and estrone sulfate were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO, USA), respectively. All other chemicals used were of the highest purity available.

Uptake experiment using Xenopus laevis oocytes expressing hOAT1 or hOAT3: pBK-CMV plasmid vectors containing cDNA of hOAT1 or hOAT3 were a kind gift from Prof. Ken-ichi Inui (Kyoto University Hospital, Kyoto, Japan). An uptake experiment using Xenopus laevis oocytes was performed as previously reported. Briefly, capped RNA encoding hOAT1 or hOAT3 was transcribed from Xbal-linearized pBK-CMV containing hOAT1 or hOAT3, respectively, with T3 RNA polymerase. After 50 nl water or cRNA (25 ng) was injected into defolliculated oocytes, the oocytes were maintained in modified Barth’s medium (88 mM NaCl, 1 mM KCl, 0.33 mM Ca(NO3)2, 0.4 mM CaCl2, 0.8 mM MgSO4, 2.4 mM NaHCO3 and 5 mM HEPES; pH 7.4) containing 50 mg/l gentamicin at 18°C. Two or three days after injection, the uptake reaction was initiated by incubating the oocytes in 500 µl uptake buffer (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2 and 5 mM HEPES; pH 7.4) with each radiolabeled compound at room temperature in the absence or presence of chlorogenic acid, caffeic acid or quinic acid for the indicated periods. The uptake reaction was terminated by adding 2 ml ice-cold uptake buffer to each well, and the oocytes were washed three times with 2 ml ice-cold buffer. After washing, each oocyte was transferred to a scintillation-counting vial and solubilized in 150 µl of 10% sodium lauryl sulfate. Two milliliters of scintillation cocktail ClearCount® were added to each well, and radioactivity was determined using a liquid scintillation counter. We performed the same uptake experiments at least two times; typical results from each experiment are presented in the Tables and Figs.

Kinetic analysis: The apparent 50% inhibitory concentrations (IC50) of caffeic acid for hOAT1 and hOAT3 were estimated by non-linear least-squares regression analysis of the competition curve with a one-compartment model according to the following equation: A = 100 × IC50/[IC50 + [I]] × B, where A is the uptake amount of p-aminobipropionate or estrone sulfate (% of control), [I] is the concentration of caffeic acid and B is the non-specific organic anion uptake (% of control). The kinetic parameters of p-aminobipropionate transport by hOAT1 and of estrone sulfate transport by hOAT3 were calculated using non-linear least-squares regression analysis.
from the following Michaelis-Menten equation: 
\[ V = \frac{V_{\text{max}} [S]}{K_m + [S]} \]
where \( V \) is the transport rate (pmol/oocyte/h), \( V_{\text{max}} \) is the maximum velocity by the saturable process (pmol/oocyte/h), \([S]\) is the concentration of \( p \)-aminohippurate or estrone sulfate (\( \mu \)M) and \( K_m \) is the Michaelis-Menten constant (\( \mu \)M).

**Effect of pretreatment with caffeic acid on hOAT1 and hOAT3:** The oocytes injected with hOAT1 or hOAT3 cRNA were preincubated in 500 \( \mu \)l of uptake buffer with or without 100 \( \mu \)M caffeic acid for 30 min. After they were washed three times with 2 ml uptake buffer at room temperature, the oocytes were incubated with \( p \)-aminohippurate or estrone sulfate, and their accumulation was determined.

**Statistical analysis:** Data were analyzed by the unpaired t-test or one-way analysis of variance followed by Dunnett’s test using GraphPad Software, San Diego, CA, USA. Differences were considered significant at \( P < 0.05 \).

**Results**

**Time-dependent uptake of \( p \)-aminohippurate by hOAT1 and of estrone sulfate by hOAT3 and the effect of caffeic acid:** First, to investigate the influence of caffeic acid on hOAT1 and hOAT3, we measured the accumulation of their typical substrates \( p \)-aminohippurate and estrone sulfate, respectively, in oocytes with or without caffeic acid. As shown in **Figure 2A**, injection of hOAT1 cRNA increased the uptake of \( p \)-aminohippurate into the oocytes, and the uptake amounts elevated time dependently. In the presence of 100 \( \mu \)M caffeic acid, the accumulation of \( p \)-aminohippurate in oocytes expressing hOAT1 was reduced to that of water-injected oocytes. In the case of hOAT3, its expression stimulated uptake of estrone sulfate, and caffeic acid also strongly inhibited the transport (**Fig. 2B**). These findings show that caffeic acid has inhibitory effects on hOAT1 and hOAT3.

**Effects of chlorogenic acid and quinic acid on uptake of \( p \)-aminohippurate by hOAT1 and of estrone sulfate by hOAT3:** Next, we evaluated the inhibitory effects of 100 \( \mu \)M chlorogenic acid and quinic acid on hOAT1 and hOAT3, and compared the effects with those of caffeic acid. As shown in **Figures 3A** and **3B**, no effect of chlorogenic acid on hOAT1 was detected, but it inhibited hOAT3 significantly. The uptake amounts of estrone sulfate in hOAT3-expressing oocytes with caffeic acid were lower than with chlorogenic acid. No influence of quinic acid on hOAT1 or hOAT3 was observed. These data indicate that the inhibitory effects of chlorogenic acid and quinic acid on hOAT1 and hOAT3 are weaker than those of caffeic acid.

**Concentration dependence of inhibitory effect of caffeic acid on hOAT1 and hOAT3:** To estimate the IC\(_{50}\) values of caffeic acid for hOAT1 and hOAT3, the dose dependence of the inhibitory effect was investigated. As illustrated in **Figures 4A** and **4B**, caffeic acid inhibited the transport of \( p \)-aminohippurate by hOAT1 and of estrone sulfate by hOAT3 dose dependently. Complete inhibition by caffeic acid was recognized at 1 \( \text{mM} \). The apparent IC\(_{50}\) values were 16.6 \( \pm \) 3.7 \( \mu \)M for hOAT1 and 5.4 \( \pm \) 1.3 \( \mu \)M for hOAT3 (mean \( \pm \) S.E.M. from three independent experiments).

**Manner of inhibition of hOAT1 and hOAT3 by caffeic acid:** To elucidate the mode of inhibition of hOAT1 and hOAT3 by caffeic acid, Eadie-Hofstee plot analysis was performed, and typical results are shown in **Figure 5**. Caffeic acid significantly increased the \( K_m \) value of
p-aminohippurate transport by hOAT1 from 4.7 ± 0.4 µM to 16.3 ± 2.1 µM (mean ± S.E.M. from three separate experiments, \( P = 0.0053 \)), and the \( V_{\text{max}} \) values were calculated to be 22.5 ± 3.6 pmol/oocyte/h and 16.4 ± 2.3 pmol/oocyte/h in the absence or presence of caffeic acid, respectively, with no statistical difference recognized between these values (\( P = 0.2275 \)). The slope of the Eadie-Hofstee plots for p-aminohippurate transport by hOAT1 was markedly affected (Fig. 5B).

The result for hOAT3 showed a similar tendency to that for hOAT1. The slope of the Eadie-Hofstee plot for the hOAT3-mediated transport of estrone sulfate was also influenced by caffeic acid (Fig. 5D). The \( K_m \) values were estimated to be 3.4 ± 0.7 µM and 9.9 ± 2.0 µM (mean ± S.E.M. from three separate experiments) in the absence or presence of caffeic acid, respectively, and the difference was statistically significant (\( P = 0.0413 \)), whilst no significant change in the \( V_{\text{max}} \) values was observed (without caffeic acid, 5.3 ± 1.7 pmol/oocyte/h; with caffeic acid, 4.7 ± 1.5 pmol/oocyte/h; \( P = 0.7858 \)). These findings imply that caffeic acid inhibited hOAT1 and hOAT3 in a competitive manner.
Effect of pretreatment with caffeic acid on hOAT1 and hOAT3: To investigate whether the inhibitory effect of caffeic acid on hOAT1 and hOAT3 is reversible, oocytes expressing hOAT1 or hOAT3 were preincubated with 100 µM caffeic acid for 30 min before incubation with p-aminohippurate or estrone sulfate, respectively. As shown in Table 1, the preincubation with caffeic acid did not influence p-aminohippurate transport by hOAT1. Also, in hOAT3, the effect on estrone sulfate uptake was not statistically recognized (Table 2). These findings suggest that inhibitory effect of caffeic acid on the transporters is reversible.

Effect of caffeic acid on transport of antifolates and antivirals by hOAT1 and hOAT3: Finally, we examined the inhibitory effects of caffeic acid on the transport of other drugs by hOAT1 and hOAT3. As shown
Table 1. Effect of pretreatment with caffeic acid on p-aminohippurate uptake by hOAT1

<table>
<thead>
<tr>
<th>Injection</th>
<th>Pretreatment</th>
<th>Uptake (fmol/oocyte/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>None</td>
<td>13.6 ± 1.0</td>
</tr>
<tr>
<td>hOAT1 cRNA</td>
<td>None</td>
<td>1088 ± 145</td>
</tr>
<tr>
<td>hOAT1 cRNA</td>
<td>Uptake buffer</td>
<td>1052 ± 197</td>
</tr>
<tr>
<td>hOAT1 cRNA</td>
<td>100 µM caffeic acid</td>
<td>1185 ± 192</td>
</tr>
</tbody>
</table>

Oocytes injected with hOAT1 cRNA were preincubated in uptake buffer in the absence or presence of 100 µM caffeic acid. After the oocytes were washed 3 times with 2 ml of uptake buffer at room temperature, they were incubated with 17.5 nM [3H]p-aminohippurate for 1 h. The uptake amounts of [3H]p-aminohippurate in each oocyte were determined. Each value represents the mean ± S.E.M. of 8 to 10 oocytes.

Table 2. Effect of pretreatment with caffeic acid on estrone sulfate uptake by hOAT3

<table>
<thead>
<tr>
<th>Injection</th>
<th>Pretreatment</th>
<th>Uptake (fmol/oocyte/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>None</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>hOAT3 cRNA</td>
<td>None</td>
<td>45.1 ± 6.0</td>
</tr>
<tr>
<td>hOAT3 cRNA</td>
<td>Uptake buffer</td>
<td>40.2 ± 6.4</td>
</tr>
<tr>
<td>hOAT3 cRNA</td>
<td>100 µM caffeic acid</td>
<td>47.6 ± 8.7</td>
</tr>
</tbody>
</table>

Oocytes injected with hOAT3 cRNA were preincubated in uptake buffer in the absence or presence of 100 µM caffeic acid. After the oocytes were washed 3 times with 2 ml of uptake buffer at room temperature, they were incubated with 17.5 nM [3H]estrone sulfate for 1 h. The uptake amounts of [3H]estrone sulfate in each oocyte were determined. Each value represents the mean ± S.E.M. of 8 to 10 oocytes.

Table 3. Effect of caffeic acid on transport of methotrexate, aminopterin, acyclovir, and penciclovir by hOAT1

<table>
<thead>
<tr>
<th>Uptake (fmol/oocyte/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-injected</td>
</tr>
<tr>
<td>Methotrexate</td>
</tr>
<tr>
<td>Aminopterin</td>
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<tr>
<td>Acyclovir</td>
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<tr>
<td>Penciclovir</td>
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</tbody>
</table>

Water-injected oocytes were incubated with 41.7 nM [3H]methotrexate, 28.5 nM [3H]aminopterin, 70.4 nM [3H]acyclovir or 94.3 nM [3H]penciclovir for 1 h. hOAT1 cRNA-injected oocytes were incubated with these radiolabeled compounds in the absence (control) or presence of 30 µM caffeic acid for 1 h. The uptake amounts of the radiolabeled compounds in each oocyte were determined. Each value represents the mean ± S.E.M. of 8 to 10 oocytes. ***P < 0.001, significantly different from the control values.

Table 4. Effect of caffeic acid on transport of methotrexate and penciclovir by hOAT3

<table>
<thead>
<tr>
<th>Uptake (fmol/oocyte/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-injected</td>
</tr>
<tr>
<td>Methotrexate</td>
</tr>
<tr>
<td>Penciclovir</td>
</tr>
</tbody>
</table>

Water-injected oocytes were incubated with 41.7 nM [3H]methotrexate or 94.3 nM [3H]penciclovir for 1 h. hOAT3 cRNA-injected oocytes were incubated with these radiolabeled compounds in the absence (control) or presence of 30 µM caffeic acid for 1 h. The uptake amounts of the radiolabeled compounds in each oocyte were determined. Each value represents the mean ± S.E.M. of 8 to 10 oocytes. ***P < 0.001, significantly different from the control values.

Discussion

The transport of substances by hOAT1 and hOAT3 in the kidney plays pharmacokinetic, pharmacological, physiological, toxicological and pathophysiological roles. Accordingly, identification of their inhibitors is useful in establishing optimal drug therapy and development of new agents. So far, the inhibitory characteristics of drugs toward hOAT1 and hOAT3 have been widely examined, but few reports have shown the regulation of these transporters by substances in the diet. At present, many food ingredients are commercially available, and it is possible to extract the factors responsible for food–drug interactions from in vitro experiments. In this study, we discovered that caffeic acid, which is contained in coffee, fruits and vegetables, strongly inhibited hOAT1 and hOAT3, and this inhibition was characterized. These transporters are considered to mediate renal tubular uptake of various drugs from blood, and we showed that the transport of antifolates and antivirals by hOAT1 and hOAT3 was inhibited by caffeic acid (Tables 3 and 4). It is thought that the inhibition of hOAT1 and hOAT3 by caffeic acid could be a novel candidate for food–drug interactions. Taken together, the results of the present study with those of experiments using renal slices or in vivo study might yield useful information to attenuate the cytotoxicity of antifolates and antivirals.

Recently, the International Transporter Consortium proposed that a clinical trial of the interactions between a substrate and an inhibitor of a transporter is necessary when the value of the unbound maximum plasma concentration of the inhibitor divided by its IC₅₀ is ≥ 0.1. On the plasma levels of caffeic acid, Kempf et al. reported that the median value was 38.3 µM when drinking four cups of coffee a day for 1 month, and that the concentration reached more than 100 µM in one-fourth of the participants drinking eight cups. The protein binding ratio of caffeic acid was estimated to be 66%. Accordingly, it is supposed that the unbound plasma concentration of caffeic acid would be above 10 µM in most participants who had more than four cups of coffee a day. Taken together with Figure 4, it is suggested that moderate to strong inhibition of hOAT1 and hOAT3 would occur in the presence of caffeic acid at

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this level, and this fulfills the condition proposed by the International Transporter Consortium. However, Renouf et al. showed that the total plasma concentration of caffeic acid was less than 100 nM after drinking 4 g instant coffee soluble in 400 ml water, meaning that this range is much lower than the inhibitory levels of hOAT1 and hOAT3. From the point of the interaction between caffeic acid and substrates of hOAT1 and hOAT3, the difference between the two reports is very interesting. Kempf et al. put forward the duration of the coffee intake as a cause of the high plasma levels of caffeic acid in their report. It is speculated that the brand of coffee may also be a reason for the difference. Elucidation of the relationship between coffee consumption and plasma levels of caffeic acid is desired to assess its effects on the pharmacokinetics of drugs transported by hOAT1 and hOAT3. Unfortunately, to our knowledge there are no reports describing the interaction of substrate drugs of hOAT1 and hOAT3 with caffeic acid or with coffee. We hope that the results of this current report will encourage research to investigate such potential interactions.

One of the unique findings in this study is the relationship between the inhibitory effects of caffeic acid, chlorogenic acid and quinic acid on hOATs, and their structures. As shown in Figure 3, 100 µM caffeic acid strongly inhibited hOAT1 and hOAT3; however, inhibition of hOAT1 and hOAT3 by quinic acid was not observed. Chlorogenic acid moderately inhibited hOAT3, but no effect on hOAT1 was found. These findings imply that the inhibitory effects of chlorogenic acid and quinic acid on the transporters are weaker than those of caffeic acid. It is interesting to compare these results with findings for NSAIDs. Most NSAIDs strongly inhibited hOAT1 and hOAT3, with IC50 values or Ki values of less than 20 µM; NSAIDs also possess a carboxyl group, a typical anionic moiety. The obtained IC50 values of caffeic acid against hOAT1 and hOAT3 are comparable to those of NSAIDs. NSAIDs have a methylene group or a methine group between the benzene ring and the carboxyl group; however, caffeic acid has a vinylene group (Fig. 1). The Ki values of salicylate were reported to be 407 µM for hOAT1 and 111 µM for hOAT3, meaning that its inhibitory effect is weaker than those of other NSAIDs. Salicylate also has a carboxyl group, but it directly binds to the benzene ring. In quinic acid and chlorogenic acid, direct binding has a carboxyl group, but it directly binds to the benzene ring. In quinic acid and chlorogenic acid, direct binding has a carboxyl group, but it directly binds to the benzene ring, including the benzene ring, may in

Acknowledgments: We thank Prof. Ken-ichi Inui (Kyoto University Hospital, Kyoto, Japan) for kindly providing pBK-CMV plasmid vectors containing hOAT1 or hOAT3.

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