A Strategy for Assessing Potential Drug-Drug Interactions of a Concomitant Agent against a Drug Absorbed via an Intestinal Transporter in Humans

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ABSTRACT

A strategy for assessing potential drug-drug interactions (DDIs) based on a simulated intestinal concentration is described. The proposed prediction method was applied to the DDI assessment of luseogliflozin, a novel anti-diabetic drug, against miglitol absorbed via the intestinal sodium-glucose cotransporter 1 (SGLT1). The method involves four steps: collection of physicochemical and pharmacokinetic parameters of luseogliflozin for use in a computer simulation; evaluation of the validity of these parameters by verifying the goodness of fit between simulated and observed plasma profiles; simulation of the intestinal luseogliflozin concentration-time profile using the Advanced Compartment Absorption and Transit (ACAT) model in a computer program and estimation of the time spent above a value 10-fold higher than the IC50 value (TAIC) for SGLT1; and evaluation of the DDI potential of luseogliflozin by considering the percentage of TAIC against the miglitol T_max (time for C_max) value (TAIC/T_max). An initial attempt to prove the validity of this method was performed in rats. The resulting TAIC/T_max in rats was 32%, suggesting a low DDI potential of luseogliflozin against miglitol absorption. The validity was then confirmed using an in vivo interaction study in rats. In humans, luseogliflozin was expected to have no DDI potential against miglitol absorption, since the TAIC/T_max in humans was lower than that in rats. This prediction was proven, as expected, in a clinical interaction study. In conclusion, the present strategy based on a simulation of the intestinal concentration-time profile using dynamic modeling would be useful for assessing the clinical DDI potential of a concomitant agent against drugs absorbed via an intestinal transporter.

INTRODUCTION

Drug-drug interactions (DDIs), which involve the inhibition/induction of drug-metabolizing enzymes or transporters, may result in adverse drug reactions and a possible loss of efficacy. For these reasons, understanding DDIs that might occur during the process of absorption, metabolism, or excretion is a key component of clinical drug development to ensure patient safety and drug efficacy, and is an integral part of the regulatory review process that must be undertaken prior to market approval. Since clinical interaction studies are expensive and time consuming, an alternative strategy for predicting the DDI potential in humans is needed. New approaches based on the latest scientific knowledge and tools can help to reduce both the cost and time required to develop and evaluate new drugs. For example, the extensive use of computer-based modeling and simulation could be a valuable tool. The prediction of DDI potential using modeling and simulation has also been mentioned in regulatory guidances published by both the U.S. Food and Drug Administration (FDA) (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362.pdf) and the European Medicines Agency (www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf), in which the assessment of DDIs using static and dynamic models, including physiologically based pharmacokinetics models, is recommended. Indeed, various prediction methods using static and dynamic models for metabolism-based DDIs have been reported, and a dynamic modeling approach appears to allow a more accurate prediction of the DDI potential than a static modeling approach (Kato et al., 2008; Fähmi et al., 2009). On the other hand, prediction methods using dynamic models for transporter-based DDI potentials during intestinal absorption have not yet been reported, with the exception of reports describing P-glycoprotein–mediated efflux (Neuhoff et al., 2013; Reyner et al., 2013). Furthermore, no methods using dynamic modeling to predict the

ABBREVIATIONS: ACAT, Advanced Compartment Absorption and Transit; AIC, Akaike information criterion; AUC0-∞, area under the curve from zero to time t; CI, confidence interval; CLR, total clearance; DDI, drug-drug interaction; FDA, U.S. Food and Drug Administration; Fh, hepatic availability; FPE, first-pass effect; hSGLT, human sodium-glucose cotransporter; [I], concentration of inhibitor in the gastrointestinal tract arising from the highest approved clinical dose dissolved in 250 ml of water; IS, internal standard; IV, intravenous; k12, rate constant for compartment 1 to 2; k21, rate constant for compartment 2 to 1; Ka, absorption rate constant; LC-MS/MS, liquid chromatography–tandem mass spectrometry; α-MG, methyl-α-D-glucopyranoside; Peff, effective permeability; PK, pharmacokinetics; SGLT, rat sodium-glucose cotransporter; SGLT1, sodium-glucose cotransporter; TAIC, time spent above a value 10-fold higher than the IC50 value; T_max, time for C_max; Vc, volume of distribution.
DDI potential against drugs absorbed via an intestinal transporter have been reported to date.

Although membrane transporters are not as well recognized as metabolizing enzymes, they can have important effects on pharmacokinetics (PK) and drug exposure (Shugarts and Benet, 2009). Therefore, a suitable approach for predicting the human DDI potential of a concomitant agent against drugs absorbed via intestinal transporters is needed.

Sodium-glucose cotransporters (SGLTs) participate in the process of glucose absorption and include SGLT1, which is largely found in small intestinal cells, as well as SGLT2, which is mainly located in the proximal tubule in the kidneys (Hediger and Rhoads, 1994; Chen et al., 2010; Hummel et al., 2011). In the current study, miglitol and luseogliflozin were adopted as model compounds for a SGLT1 substrate (Kuboyama et al., 2006; Pharmaceuticals and Medical Devices Agency, Japan, http://www.info.pmda.go.jp/shinyaku/P200500031/index.html) and a concomitant drug, respectively. These compounds are used in combination during clinical treatment. Miglitol, an α-glucosidase inhibitor, is absorbed via SGLT1 and is a widely prescribed drug for the treatment of type 2 diabetes mellitus; miglitol acts by influencing carbohydrate digestion to blunt the postprandial blood glucose increase (Sels et al., 1999). Luseogliflozin is a novel and potent selective SGLT2 inhibitor (Kakinuma et al., 2010; Suzuki et al., 2012; Washburn and Poucher, 2013) that is orally available for the treatment of type 2 diabetes. Selective SGLT2 inhibitors for type 2 diabetes are now receiving special attention because of their novel and safe mechanisms of action. SGLT2 is responsible for 90% of glucose reabsorption (Hediger and Rhoads, 1994; Pérez López et al., 2010), and the inhibition of SGLT2 leads to a decrease in blood glucose through an increase in the renal excretion of excess glucose. Almost all selective SGLT2 inhibitors have a common basic structure similar to that of phlorizin, a natural nonselective SGLT inhibitor that has long been known to increase glucose excretion into the urine and to reduce the blood glucose level in diabetic animals (Khan and Efendic, 1995; Krook et al., 1997). These compounds also have the potential to inhibit SGLT1 slightly. Although luseogliflozin has a relatively high selectivity for SGLT2 compared with similar kinds of drugs (Suzuki et al., 2012), the value of \( [I_2] \) represents the concentration of inhibitor in the gastrointestinal tract arising from the highest approved clinical dose (5 mg) dissolved in 250 ml of water, was much higher than the 50% inhibitory concentration (IC50) value for SGLT1 (SGLT IC50). The value of \([I_2]/SGLT1\) IC50 was higher than 10, which is the value used as a decision criterion for performing a clinical DDI study as mentioned in the current FDA DDI draft guidance.

The objective of this study was to propose a strategy for assessing the clinical DDI potential of a concomitant agent against a drug that is absorbed via an intestinal transporter by performing a DDI assessment of luseogliflozin against miglitol.

**Materials and Methods**

Luseogliflozin(1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol was synthesized at Taisho Pharmaceutical Co., Ltd. (Saitama, Japan). Miglitol and phlorizin were purchased from Toronto Research Chemicals Inc. (North York, Ontario, Canada). Stable isohte-labeled luseogliflozin (\(^{12}H_2\)-luseogliflozin), which was used as an internal standard (IS) for the quantitative analysis of both luseogliflozin and phlorizin, and stable isotope-labeled miglitol (\(^{2}H_4\)-miglitol), which was used as an IS for the quantitative analysis of miglitol, were synthesized at Taisho Pharmaceutical Co., Ltd. The chemical structures of luseogliflozin, miglitol, and phlorizin are shown in Fig. 1. Methyl-\(\alpha\)-D-glucopyranoside (\(\alpha\)-MG) and \(^{13}C\)\(\alpha\)-MG were purchased from Sigma-Aldrich (St. Louis, MO) and PerkinElmer (Tokyo, Japan), respectively. Blank rat plasma was obtained from Charles River Laboratories Japan (Kanagawa, Japan).

**Animals**

Eight-week-old male Sprague-Dawley rats (Charles River Laboratories Japan) were used for the experiments. All of the experimental procedures involving animal handling were approved by the Institutional Animal Care and Use Committee of Taisho Pharmaceutical Co., Ltd., and were in accordance with the Guidelines for the Proper Conduct of Animal Experiments (Science Council of Japan, 2006, http://www.scj.go.jp/en/report/index.html).

**Determination of IC50 Value for SGLT1**

Chinese hamster ovary-K1 cells were obtained from the American Type Culture Collection (Rockville, MD) and were stably transfected with a plasmid vector for human SGLT1 (hSGLT1; GenBank accession number NM_000343). The cells were cultured in a 96-well plate in F-10 Nutrient Mixture (Life Technologies Co., Ltd., Carlsbad, CA) containing 10% fetal bovine serum and 250 μg/ml hygromycin for 2 days. African green monkey SV40-transfected kidney fibroblast cells (COS-7) obtained from the American Type Culture Collection were seeded into a 96-well plate in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum 1 day before transfection. Then the cells in each well were transiently transfected individually with a plasmid vector for rat SGLT1 (rSGLT1; GenBank accession number NM_013033) and cultured for 1 day. The cells expressing rSGLT1 or hSGLT1 were incubated in a pretreatment buffer (140 mM choline chloride, 2 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM HEPES, 5 mM Tris, pH 7.2-7.4) at 37°C for 20 minutes. Then the cells were incubated in an uptake buffer [Na+] (140 mM NaCl, 2 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM HEPES, 5 mM Tris, pH 7.2-7.4) containing an \(\alpha\)-MG substrate mixture ([\(^{13}C\)\(\alpha\)-MG and \(\alpha\)-MG) and various concentrations of the test agents or the vehicle alone (dimethylsulfoxide) at 37°C for 20 or 30 minutes for the rSGLT1 or hSGLT1 assay, each of which was performed in triplicate. The concentration of the \(\alpha\)-MG substrate mixture was 500 μM and 1 mM for the rSGLT1 and hSGLT1 assays, respectively. The uptake reaction was terminated by washing the cells twice with the pretreatment buffer containing 10 mM \(\alpha\)-MG, and the cells were then lysed in 0.25 M NaOH. Radioactivity was measured using a liquid scintillation counter. The sodium-independent uptake was measured in a sodium-free uptake buffer [Na+] (140 mM choline chloride, 2 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM HEPES, 5 mM Tris, pH 7.2-7.4) containing an \(\alpha\)-MG substrate mixture ([\(^{13}C\)\(\alpha\)-MG and \(\alpha\)-MG, 500 μM for rSGLT1, 1 mM for hSGLT1) and dimethylsulfoxide as the vehicle. The sodium-dependent \(\alpha\)-MG uptake was calculated by subtracting the sodium-independent uptake count from each count measured in the uptake buffer.

**Fig. 1.** Chemical structures of luseogliflozin (A), phlorizin (B), and miglitol (C).
## Table 1

<table>
<thead>
<tr>
<th>Property</th>
<th>Luseogliflozin in Rats</th>
<th>Phlorizin in Rats</th>
<th>Luseogliflozin in Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Input Data</td>
<td>Reference/Remarks</td>
<td>Input Data</td>
</tr>
<tr>
<td><strong>Physicochemical parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular formula</td>
<td>$C_{23}H_{30}O_6S$</td>
<td>—</td>
<td>$C_{21}H_{24}O_{10}$</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>434.55</td>
<td>—</td>
<td>436.42</td>
</tr>
<tr>
<td>Reference logP (pH)</td>
<td>2.2 (-1)</td>
<td>In-house data</td>
<td>0.25 (-1)</td>
</tr>
<tr>
<td>Dosage form</td>
<td>IR: solution</td>
<td>—</td>
<td>IR: suspension</td>
</tr>
<tr>
<td>Initial dose (mg)</td>
<td>0.03</td>
<td>—</td>
<td>12</td>
</tr>
<tr>
<td>Dose volume (ml)</td>
<td>2</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Solubility (mg/ml at pH = 6.57)</td>
<td>0.0771</td>
<td>In-house data</td>
<td>9.27 (mg/ml at pH = 4.49)</td>
</tr>
<tr>
<td>Diff. coeff. (cm^2/s × 10^3)</td>
<td>0.60</td>
<td>Estimated by ADMET Predictor</td>
<td>0.64</td>
</tr>
<tr>
<td>Mean particle radius (μm)</td>
<td>3.03</td>
<td>In-house data</td>
<td>25.0</td>
</tr>
<tr>
<td>$P_{ae}$ (cm/s × 10^3)</td>
<td>6.23</td>
<td>Estimated from Ka</td>
<td>0.558</td>
</tr>
<tr>
<td><strong>Gut physiology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physiology</td>
<td>Rat-fasted</td>
<td>The stomach transit time was changed to 0.1 h.</td>
<td>Rat-Fasted</td>
</tr>
<tr>
<td><strong>Pharmacokinetic parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>0.30</td>
<td>—</td>
<td>0.30</td>
</tr>
<tr>
<td>FPE intestinal</td>
<td>45.7</td>
<td>Estimated</td>
<td>98.8</td>
</tr>
<tr>
<td>FPE liver</td>
<td>73.3</td>
<td>Estimated</td>
<td>65.4</td>
</tr>
<tr>
<td>Blood/plasma concentration ratio</td>
<td>0.536</td>
<td>In-house data</td>
<td>0.930</td>
</tr>
<tr>
<td>Fup (%)</td>
<td>5.40</td>
<td>In-house data</td>
<td>6.71</td>
</tr>
<tr>
<td>Renal clearance (l/h/kg)</td>
<td>0</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Model</td>
<td>2-compartment</td>
<td>2-compartment</td>
<td>2-compartment</td>
</tr>
<tr>
<td>CL (l/h)</td>
<td>0.495</td>
<td>Fitted from 0.1 mg/kg iv data</td>
<td>0.766</td>
</tr>
<tr>
<td>$V_c$ (l/kg)</td>
<td>1.21</td>
<td>0.877</td>
<td>0.588</td>
</tr>
<tr>
<td>$k_{12}$ (l/h)</td>
<td>0.384</td>
<td>0.0352</td>
<td>0.384</td>
</tr>
<tr>
<td>$k_{21}$ (l/h)</td>
<td>0.271</td>
<td>0.535</td>
<td>0.271</td>
</tr>
</tbody>
</table>

ASF, absorption scaling factor; Diff. coeff., diffusion coefficient; Fup, plasma unbound fraction; IR, immediate release; po, oral.
Estimation of the Time Spent above a Value 10-Fold Higher than the IC50 Value in the Intestine Using GastroPlus

The concentrations of luseogliflozin and phlorizin (positive control) in the plasma and intestine were simulated using GastroPlus version 8.0 (Simulation Plus Inc., Lancaster, CA). This simulator is an advanced-technology computer program that simulates gastrointestinal absorption, distribution, and the PK of agents dosed via an oral route in humans and animals. The underlying model in GastroPlus is the Advanced Compartment Absorption and Transit (ACAT) (Agoram et al., 2001). The physiologically based ACAT model consists of nine compartments corresponding to different segments of the digestive tract, and is based on the original compartmental absorption and transit model described by Yu et al. (1996).

The time spent above a value 10-fold higher than the IC50 value (TACIC) in the intestine was estimated for both luseogliflozin and phlorizin using the procedure described in the following sections. The parameters for the computer simulation are shown in Table 1.

Estimation of Effective Permeability Values. The plasma concentration-time profiles of luseogliflozin after oral administration in rats and humans were analyzed based on a two-compartment model using the PKPlus module in GastroPlus to estimate each absorption rate constant (Ka) value. The Ka value was analyzed based on a two-compartment model using the PKPlus module in GastroPlus version 8.0 (Simulation Plus Inc., Lancaster, CA). This simulator is an advanced-technology computer program that simulates gastrointestinal absorption, distribution, and the PK of agents dosed via an oral route in humans and animals. The underlying model in GastroPlus is the Advanced Compartment Absorption and Transit (ACAT) (Agoram et al., 2001). The physiologically based ACAT model consists of nine compartments corresponding to different segments of the digestive tract, and is based on the original compartmental absorption and transit model described by Yu et al. (1996).

The time spent above a value 10-fold higher than the IC50 value (TACIC) in the intestine was estimated for both luseogliflozin and phlorizin using the procedure described in the following sections. The parameters for the computer simulation are shown in Table 1.

Estimation of Fraction Absorbed Value and PK Parameters. The fraction absorbed value was predicted by inputting the compound structure and inputting the related parameters (dosage form, dose, solubility, diffusion coefficient, particle radius, and permeability) using GastroPlus. The transit time for the stomach was set as 0.1 hour based on the recommended value, since the dosage form was not a tablet, but a solution or suspension. The PK parameters (total clearance (CL), volume of distribution (Vd), rate constant for compartment 1 to 2 (k12) and compartment 2 to 1 (k21)) of luseogliflozin and phlorizin in rats were obtained by fitting the iv plasma concentration-time data using the PKPlus module, and the parameters (CL/bioavailability (F), Vc/F, k12, k21) of luseogliflozin in humans were obtained by fitting the plasma concentration-time data after oral administration using the PKPlus module. During this fitting process, the best compartment model was selected according to the Akaike information criterion (AIC), which indicated that the minimum AIC value was the best representation of the model (Yamaoka et al., 1978).

Estimation of Hepatic and Intestinal First-Pass Effect. The hepatic and intestinal first-pass effects (FPEs) were estimated as follows:

\[
P_{\text{eff}} = Ka \times \frac{V}{S} = Ka \times \frac{r}{2}
\]

where V, S, and r are the luminal volume, surface area, and luminal radius, respectively.

The values for r were 0.18 cm for rats (Komiya et al., 1980) and 1.75 cm for humans (Fagerholm et al., 1996)

Estimation of Fraction Absorbed Value and PK Parameters. The fraction absorbed value was predicted by inputting the compound structure and inputting the related parameters (dosage form, dose, solubility, diffusion coefficient, particle radius, and permeability) using GastroPlus. The transit time for the stomach was set as 0.1 hour based on the recommended value, since the dosage form was not a tablet, but a solution or suspension. The PK parameters (total clearance (CL), volume of distribution (Vd), rate constant for compartment 1 to 2 (k12) and compartment 2 to 1 (k21)) of luseogliflozin and phlorizin in rats were obtained by fitting the iv plasma concentration-time data using the PKPlus module, and the parameters (CL/bioavailability (F), Vc/F, k12, k21) of luseogliflozin in humans were obtained by fitting the plasma concentration-time data after oral administration using the PKPlus module. During this fitting process, the best compartment model was selected according to the Akaike information criterion (AIC), which indicated that the minimum AIC value was the best representation of the model (Yamaoka et al., 1978).

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where Fh is the hepatic availability and Fg is the intestinal availability. The hepatic clearance was assumed to equal to the CL with a negligible contribution from renal clearance. The Fh and Fg values for luseogliflozin and phlorizin in rats were estimated based on the following equations:

\[
Fh = 1 - \frac{Qh}{(CL/Rb)}
\]

\[
Fg = \frac{F}{(Fa \times Fh)}
\]

where a hepatic blood flow (Qh) value of 4.2 l/h/kg (Hosea et al., 2009) was used. The FPE values of luseogliflozin in humans were set at zero, because the PK parameters (CL/F and Vc/F) of luseogliflozin in humans involved these related parameters.

Estimation of TAIC in Intestine. The validity of the estimated parameters shown in Table 1 was confirmed by verifying the goodness-of-fit between the simulated and observed plasma profiles. Then a simulation was performed to assess the DDI potential. In rats, the concentrations in the duodenum were simulated, since SGLT1 is mainly expressed in the small intestine (Lee et al., 1994) and miglitol is almost completely absorbed via the duodenum (Pharmaceuticals and Medical Devices Agency, Japan, http://www.info.pmda.go.jp/shinyaku/P20050030/index.html). The TAIC value of luseogliflozin and phlorizin were then estimated. In humans, the concentrations in the duodenum, jejunum1, jejunum2, ileum1, ileum2, and ileum3 were simulated, since SGLT1 is known to be mainly expressed in the small intestine (Hediger and Rholuds, 1994), but no further data were available.

PK and Interaction Study in Rats

The dose regimens and formulations are summarized in Table 2. The oral dosing concentrations of miglitol and luseogliflozin for male rats were set based on each clinical dose regimen, whereas that of phlorizin (positive control) was set at an excessive concentration that was expected to inhibit SGLT1 in the intestine completely. Animals were housed in a cage on the day preceding and after administration. The compounds were administered intravenously to the jugular vein while anesthesia was maintained with 5% isoflurane (Mylan Inc., Canonsburg, PA) and a total O2 flow rate of 0.5–1 l/min using an R2 Rodent Anesthesia System (VetEquip, Inc., Pleasanton, CA). Alternatively, the compounds were administered orally using a gastric tube. Blood samples were collected from the caudal vein at 5, 15, and 30 minutes and at 1, 2, 4, 8, and 24 hours after dosing. The plasma samples were obtained from the blood by centrifugation and were stored at −80°C until sample preparation for the liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis.

Clinical PK and Interaction Study

In the clinical PK study, miglitol (50-mg tablet) or luseogliflozin (5-mg tablet) was orally administered immediately before breakfast on the day of drug administration in 12 subjects (healthy Japanese adult males), and the PK parameters were then obtained. Clinical interaction studies were conducted to determine the PK of a single oral dose of miglitol when administered in combination with

**TABLE 2**

Dose regimens and formulations used in the pharmacokinetic and interaction studies in rats

<table>
<thead>
<tr>
<th>Study</th>
<th>Compound</th>
<th>Route</th>
<th>N</th>
<th>Dose (mg/kg)</th>
<th>Conc. (mg/ml)</th>
<th>Vehicle</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacokinetics</td>
<td>Luseogliflozin</td>
<td>iv</td>
<td>3</td>
<td>0.1</td>
<td>0.1</td>
<td>10% HP-β-CD</td>
<td>Solution</td>
</tr>
<tr>
<td></td>
<td>Phlorizin</td>
<td>iv</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>PEG400/saline (4:6:v/v)</td>
<td>Solution</td>
</tr>
<tr>
<td></td>
<td>Luseogliflozin</td>
<td>po</td>
<td>6</td>
<td>0.1</td>
<td>0.015&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5% CMC-Na</td>
<td>Solution</td>
</tr>
<tr>
<td></td>
<td>Phlorizin</td>
<td>po</td>
<td>6</td>
<td>40</td>
<td>6</td>
<td>0.5% CMC-Na</td>
<td>Suspension</td>
</tr>
<tr>
<td></td>
<td>Miglitol alone&lt;sup&gt;b&lt;/sup&gt;</td>
<td>po</td>
<td>6</td>
<td>1.5</td>
<td>0.225&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5% CMC-Na</td>
<td>Solution</td>
</tr>
<tr>
<td></td>
<td>Combination</td>
<td>Miglitol</td>
<td>po</td>
<td>6</td>
<td>1.5</td>
<td>0.225&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5% CMC-Na</td>
</tr>
<tr>
<td></td>
<td>Luseogliflozin</td>
<td>po</td>
<td>6</td>
<td>0.1</td>
<td>0.015&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5% CMC-Na</td>
<td>Solution</td>
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<tr>
<td></td>
<td>Combination</td>
<td>Miglitol</td>
<td>po</td>
<td>6</td>
<td>1.5</td>
<td>0.225&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5% CMC-Na</td>
</tr>
<tr>
<td></td>
<td>Phlorizin</td>
<td>po</td>
<td>6</td>
<td>40</td>
<td>6</td>
<td>0.5% CMC-Na</td>
<td>Suspension</td>
</tr>
</tbody>
</table>

CMC-Na, carboxymethylcellulose sodium; Conc., concentration; 10% HP-β-CD, 10% hydroxypropyl β-cyclodextrin; N, number of animals; PEG400, polyethylene glycol 400; po, oral.

<sup>a</sup>The concentration values were set based on the clinical dose regimens.

<sup>b</sup>Referred from Murano-Yashira et al., 2014.
luseogliflozin. Blood samples obtained during both studies were collected preadministration and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after administration. The plasma samples were obtained from the blood by centrifugation and were stored at −80°C until sample preparation for the LC-MS/MS analysis.

Estimation of the TAIC/T\text{max}

The percentage of luseogliflozin or phlorizin TAIC in the intestine versus the miglitol time for C\text{max} (T\text{max}) value (TAIC/T\text{max}), which was regarded as the DDI potential index in the present study, was estimated using the following equation:

\[
\text{TAIC/T\text{max}} = \frac{\text{TAIC}}{\text{T\text{max}}} \times 100(\%) \quad (4).
\]

Sample Preparation for LC-MS/MS Analysis

Miglitol in Rat Plasma. Twenty microliters of IS solution (25 ng/ml) and 200 μl of acetonitrile/28% ammonium solution (98:2, v/v) were added to 50 μl of rat plasma sample, and the samples were vortexed and centrifuged. The supernatant was then collected and applied to a solid phase extraction cartridge (Mono Spin C18-CX; GL Science, Tokyo, Japan). Miglitol and the IS were eluted by centrifugation at preset values: 10,600 g, 4°C, 1 minute. A 5-μl aliquot of the eluate was then injected into the LC-MS/MS system (Mizuno-Yasuhira et al., 2014).

Luseogliflozin and Phlorizin in Rat Plasma. Twenty-five microliters of IS solution (100 ng/ml) and 500 μl of 10 mM ammonium acetate solution were added to 50 μl of rat plasma sample, and the samples were vortexed. The samples were then loaded into Oasis HLB cartridges (30 mg/1 cc; Waters, Milford, MA), washed, and then eluted with 1 ml of methanol/acetic acid (100:0.1, v/v) by centrifugation (preset values: 200 g, 4°C, 1 minute). The eluate was evaporated to dryness and dissolved in 100 μl of acetonitrile/10 mM ammonium acetate solution (20:80, v/v). A 15-μl aliquot of the filtrate was then injected into the LC-MS/MS system.

Miglitol in Human Plasma. Thirty microliters of IS solution (500 ng/ml) and 500 μl of acetonitrile/distilled water (80:20, v/v) were added to 50 μl of rat plasma sample, and the samples were vortexed and centrifuged. The supernatant was then collected and applied to a solid phase extraction cartridge (Mono Spin C18-CX; GL Science, Tokyo, Japan). Miglitol and the IS were eluted by centrifugation at preset values: 10,600 g, 4°C, 1 minute. A 5-μl aliquot of the eluate was then injected into the LC-MS/MS system (Mizuno-Yasuhira et al., 2014).

Luseogliflozin and Phlorizin in Rat Plasma. Twenty-five microliters of IS solution (100 ng/ml) and 500 μl of 10 mM ammonium acetate solution were added to 50 μl of rat plasma sample, and the samples were vortexed and centrifuged. The supernatant was then collected and applied to a solid phase extraction cartridge (Mono Spin C18-CX; GL Science, Tokyo, Japan). Miglitol and the IS were eluted by centrifugation at preset values: 10,600 g, 4°C, 1 minute. A 5-μl aliquot of the eluate was then injected into the LC-MS/MS system (Mizuno-Yasuhira et al., 2014).

Simulated luminal concentration-time profiles in rat duodenum (red line) and TAIC after the single oral administration of luseogliflozin (0.1 mg/kg) (A) and phlorizin (40 mg/kg) (B). The dashed lines represent a value 10-fold higher than the IC\text{50} value for SGLT1. The TAIC of luseogliflozin in rats (9 minutes) was shorter than the T\text{max} of miglitol in rats (28 minutes). On the other hand, the TAIC of phlorizin in rats (75 minutes) was longer.
human plasma sample, and the samples were vortexed and centrifuged. After the supernatant was collected, 810 µl of distilled water and 2 ml of chloroform were added. The mixture was shaken (approximately 230 rpm, 5 minutes) and centrifuged (preset values: 400 × g, 4°C, 5 minutes). Nine hundred microliters of acetonitrile was then added to 100 µl of the supernatant, and the sample was vortexed. A 10-µl aliquot of the mixture was then injected into the LC-MS/MS system.

**LC-MS/MS Conditions**

**Miglitol, Luseogliflozin, and Phlorizin in Rat Plasma.** The LC-MS/MS system consisted of a Shimadzu LC-20AD (Shimadzu, Tokyo, Japan) and TripleQuad5500 mass spectrometer (AB SCIEX, Foster City, CA). The data were collected and processed using Analyst 1.6 software (AB SCIEX, Foster City, CA). Miglitol was analyzed using an XBridge Amide column (4.6 mm i.d. × 50 mm, 3.5-µm particle size; Waters) with 10 mM ammonium acetate and acetonitrile/methanol (90:10, v/v) as the mobile phase under a gradient condition. Luseogliflozin and phlorizin were analyzed using an Inertsil ODS-3 column (2.1 mm i.d. × 50 mm, 3.5-µm particle size; GL Science) with 1 mM ammonium acetate and acetonitrile as the mobile phase under a gradient condition. The selected reaction monitoring transitions were as follows: miglitol, m/z 208 → m/z 146; luseogliflozin, m/z 433 → m/z 104; and phlorizin, m/z 435 → m/z 273.

**Miglitol in Human Plasma.** The LC-MS/MS system consisted of an Alliance 2795 separation module (Waters) and API4000 mass spectrometer (AB SCIEX). The data were collected and processed using Analyst 1.4.2 software. Miglitol in human plasma was analyzed under the same conditions as those used for rat plasma, as described earlier.

**Statistical Analysis**

The PK parameters were analyzed using Phoenix WinNonlin software, version 6.1 (Pharsight Co., Mountain View, CA), using a noncompartmental analysis. The effects of luseogliflozin and phlorizin on the PK of miglitol were assessed by analyzing the two-sided 90% confidence intervals (CIs) for the ratios of the geometric means for the PK parameters (Cmax and area under the curve from zero to time t (AUC0-t)) between miglitol alone and the combination groups. The ratio of the geometric mean and its 90% CI was estimated using an analysis of variance model with treatment as a fixed effect and with the subject as a random effect using a SAS mixed procedure (version 9.2; SAS Institute, Inc.). Bioequivalence was concluded if the 90% CIs of the ratio (combination/miglitol alone) were entirely contained within 0.8–1.25.

**Results**

**Determination of IC50 Value for SGLT1 in Rats and Humans.** The inhibitory effects of luseogliflozin and phlorizin on SGLT1 activity were evaluated by measuring the sodium-dependent uptake of α-MG into cells expressing rSGLT1 or hSGLT1. Luseogliflozin inhibited sodium-dependent α-MG uptake in cells expressing rSGLT1 with an IC50 of 895 nM, but was less potent for hSGLT1 (IC50 value: 2900 nM). Phlorizin also inhibited sodium-dependent α-MG uptake with an IC50 of 631 nM in cells expressing rSGLT1.

**Estimation of the TAIC Using GastroPlus after the Oral Administration of Luseogliflozin or Phlorizin in Rats.** The physicochemical and PK parameters of luseogliflozin and phlorizin in rats that were used in the GastroPlus computer simulation are summarized in Table 1. The Peff values of luseogliflozin and phlorizin in rats estimated from each Ka value were 6.23 cm/s × 104 and 0.558 cm/s × 104, respectively. The values of the other physicochemical parameters were estimated using ADMET Predictor (Simulation Plus Inc., Lancaster, CA) or were observed. The PK parameters (CL, Vc,k 12,k 21) in rats were estimated by fitting the observed data after intravenous administration to the compartment model using the PKPlus module in GastroPlus. For both compounds, as shown in Fig. 2 (A-1 and B-1), the two-compartment PK model provided the best fit for the data as assessed using the AIC. Based on these estimated physicochemical and PK parameters listed in Table 1, the plasma concentration-time profiles after the oral administration of luseogliflozin (0.1 mg/kg) and phlorizin (40 mg/kg) were simulated using the ACAT model in GastroPlus. As a result, as shown in Fig. 2 (A-2 and B-2), the simulated plasma concentration-time profiles of luseogliflozin and phlorizin were confirmed to be substantially superimposed on each of the observed concentrations, since the percent prediction of the error values simulated/observed of each Cmax and AUC0-t were within ±20%. Incidentally, from the results of these intravenous and oral studies, the bioavailabilities (F) of luseogliflozin and phlorizin were estimated to be 14.5% and 0.4%, respectively.

As mentioned earlier, the plasma concentration profiles of luseogliflozin and phlorizin in rats were confirmed to be well reproduced using the ACAT model. Then the TAIC of luseogliflozin or phlorizin was estimated from each luminal concentration-time profile predicted using the ACAT model and the value that was 10-fold higher than the IC50 value for SGLT1, as shown in Fig. 3. The estimated TAIC values of luseogliflozin and phlorizin in rats were 9 and 75 minutes, respectively.

**Prediction of the DDI Potential in Rats by Considering the Percentage of TAIC against the Miglitol Tmax Value (TAIC/Tmax).** The Tmax of miglitol after oral administration to rats was 28 minutes,
as determined in our previous report (Mizuno-Yasuhira et al., 2014). The TAIC/T_{max} of luseogliflozin against miglitol absorption in rats was 32%, suggesting a low DDI potential of luseogliflozin against miglitol absorption. On the other hand, the TAIC/T_{max} of phlorizin (positive control) was greater than 100% (268%), suggesting a high DDI potential (Table 3).

**Interaction Study in Rats for Verification.** Figure 4 shows the results of the interaction study for miglitol (1.5 mg/kg) in combination with luseogliflozin (0.1 mg/kg) or phlorizin (40 mg/kg) in rats. The mean plasma concentration-time profiles of miglitol after the oral administration of miglitol alone (Mizuno-Yasuhira et al., 2014) or in combination with luseogliflozin were virtually superimposable (Fig. 4A). Luseogliflozin had no effect on the miglitol PK parameters (C_{max}, AUC_{0-\infty}), with the 90% CIs for the ratios of the PK parameters falling within the bioequivalence range of 0.8–1.25 (Table 4). On the other hand, the mean plasma concentration-time profiles of miglitol after the administration of miglitol alone or in combination with phlorizin were not superimposable: the concentrations of miglitol until 1 hour after the coadministration of miglitol and phlorizin were lower than after the administration of miglitol alone (Fig. 4B). The 90% CI for the ratios of the C_{max} (combination of miglitol and phlorizin/miglitol alone) was from 0.23 to 0.72, which fell outside the lower limit of the range of 0.8–1.25. Those for the AUC_{0-\infty} ranged from 0.38 to 1.02 (Table 4). Thus, phlorizin affected the miglitol PK profile after combined oral administration.

**Estimation of TAIC Using GastroPlus after the Oral Administration of Luseogliflozin in Humans.** The TAIC of luseogliflozin in humans was estimated in a manner similar to that used for rats. The parameters for computer simulation are summarized in Table 1. The P_{eff} value of luseogliflozin in humans was 27.5 cm/s \times 10^5. The PK parameters (CL/F, V_{c}/F, k_{12}, k_{21}) in humans were estimated by fitting the observed data after oral administration to a two-compartment model (Fig. 5A-1), which produced the best fit for the data as assessed using the AIC. Then the plasma concentration-time profile of luseogliflozin (5 mg/individual) was simulated using the ACAT model in GastroPlus. As in the case for rats, as shown in Fig. 5A-2, the plasma concentration profile of luseogliflozin in humans was found to be well reproduced using the ACAT model. Then the TAIC of luseogliflozin was estimated from the luminal concentration-time profiles predicted using the ACAT model and a value 10-fold higher than the IC_{50} value for SGLT1, as shown in Fig. 6. The estimated TAIC of luseogliflozin in humans was 14 minutes.

**Prediction of the DDI Potential in Humans by Considering the Percentage of TAIC against the Miglitol T_{max} Value (TAIC/T_{max}).** The T_{max} of miglitol after oral administration in humans was 83 minutes (Fig. 7). The TAIC/T_{max} of luseogliflozin against miglitol absorption in humans was 17%. The TAIC/T_{max} value in humans was lower than that in rats, suggesting that luseogliflozin has no DDI potential against miglitol absorption in humans (Table 3).

**Clinical Interaction Study for Verification.** Figure 7 shows the results of an interaction study for miglitol (50 mg/individual) in combination with luseogliflozin (5 mg/individual) in humans. The mean plasma concentration-time profiles of miglitol after a single oral administration of miglitol alone or in combination with luseogliflozin were virtually superimposable. Luseogliflozin had no effect on the miglitol PK parameters.

<table>
<thead>
<tr>
<th>Combination</th>
<th>Parameter</th>
<th>Ratio of Geometric Mean</th>
<th>90% CI (Combination/Miglitol Alone)</th>
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</thead>
<tbody>
<tr>
<td>Miglitol and luseogliflozin</td>
<td>C_{max}</td>
<td>0.97</td>
<td>0.85 1.09</td>
</tr>
<tr>
<td></td>
<td>AUC_{0-\infty}</td>
<td>1.12</td>
<td>1.04 1.20</td>
</tr>
<tr>
<td>Miglitol and phlorizin</td>
<td>C_{max}</td>
<td>0.41*</td>
<td>0.23* 0.72*</td>
</tr>
</tbody>
</table>
|                           | AUC_{0-\infty} | 0.63*                 | 0.38* 1.02                          *The 90% CIs of the ratio (combination/miglitol alone) was outside the range of 0.8–1.25.

Fig. 5. Plasma concentration-time profiles for luseogliflozin in humans. The closed circles represent the mean observed data +/-S.D. (n = 12). (A-1) Two-compartment model-fitted profiles in humans after the single intravenous administration of luseogliflozin (5 mg/individual). The solid line was fitted using a nonlinear least-squares regression analysis. The PK parameters (CL/F, V_{c}/F, k_{12}, k_{21}) in humans were estimated by fitting the luminal concentrations obtained by this fitting to the observed data. (A-2) Simulated concentration-time profiles in humans after the single oral administration of luseogliflozin (5 mg/individual). The solid lines represent the model-simulated profiles, which were obtained using the ACAT model in GastroPlus.
accurate assessments. The Tmax is easy to monitor and was set as the evaluation of the DDI potential by considering the TAIC/Tmax. The model in GastroPlus and estimation of the TAIC, and step 4: the intestinal luseogliflozin concentration-time profile using the ACAT simulated and observed plasma profiles, step 3: the simulation of the luseogliflozin for use in a computer simulation, step 2: the evaluation of step 1: the collection of physicochemical and PK parameters of absorbed via intestinal SGLT1.

Discussion

It is important to understand the nature and magnitude of DDIs because an unexpected PK profile arising from a DDI often causes an insufficient efficacy or unfavorable side effects. In the present study, a novel prediction method using a dynamic model to examine the DDI potential of a concomitant agent against a drug absorbed via an intestinal transporter was proposed. Notably, the proportion of the inhibition duration of a concomitant agent against the absorption duration of a drug, identified as TAIC/Tmax, was considered to be important for accurate assessments. The Tmax is easy to monitor and was set as the absorption duration of a drug, whereas the TAIC was set as the duration of the submaximal inhibition of the concomitant agent. In this study, the proposed prediction method was applied to predict the DDI potential of luseogliflozin, a novel antidiabetic drug, against miglitol, which is proposed prediction method was applied to predict the DDI potential of the submaximal inhibition of the concomitant agent. In this study, the proposed prediction method was applied to predict the DDI potential of luseogliflozin, a novel antidiabetic drug, against miglitol, which is absorbed via intestinal SGLT1.

The proposed prediction method can be summarized in four steps: step 1: the collection of physicochemical and PK parameters of luseogliflozin for use in a computer simulation, step 2: the evaluation of the validity of these parameters by verifying the goodness of fit between simulated and observed plasma profiles, step 3: the simulation of the intestinal luseogliflozin concentration-time profile using the ACAT model in GastroPlus and estimation of the TAIC, and step 4: the evaluation of the DDI potential by considering the TAIC/Tmax. The validity of this evaluation was verified by matching the results with those of an in vivo interaction study. The key points of the present study can be summarized as follows. First, the computer simulation was conducted using a dynamic model, which allowed a more accurate assessment of the DDI potential in the intestine than a static model. A static model was thought to be problematic because of the potential for false-positive results (Kato et al., 2008) arising from the assumption that the maximum concentration of luseogliflozin in the intestine is persistent. Second, the simulated luminal concentration in the intestine was considered to be equal to the concentration of luseogliflozin at the binding site of SGLT1, since SGLT1 is expressed in the brush-border membrane (Hediger and Rhoads, 1994). And third, the dosing concentration of luseogliflozin in rats was set based on the clinical dose regimen (5 mg/250 ml) for the subsequent accurate prediction of the DDI potential in humans.

In addition, the following important points regarding the process used to develop the prediction method should be noted. The simulated concentration-time profiles using GastroPlus did not fit the observed data at step 2 when the simulation was conducted using the Peff value predicted by the built-in ADMET Predictor at step 1. The absorption velocity of the simulated concentration-time profiles seemed to be lower than the observed concentration as a result of the low Peff value. The observed Tmax values of luseogliflozin and phlorizin were 0.25 hour each, but the simulated Tmax values were 0.64 and 1.27 hours, respectively. Furthermore, the simulated Cmax value of phlorizin also deviated from that of the observed data. Therefore, referring to a report by Amidon et al. (1995), the Peff values in rats were estimated based on each experimental Ka value using eq. 1. Using these Peff values, the simulated concentration-time profiles and the observed values were well matched, and the Tmax values of luseogliflozin and phlorizin improved to 0.37 and 0.51 hour, respectively (Fig. 2, A-2 and B-2). Under these conditions, the PK of luseogliflozin and of phlorizin in rats was confirmed to be well reproduced using the ACAT model. In addition, the sensitivity analyses were performed using GastroPlus to confirm the effective parameters for the Tmax of luseogliflozin and the Tmax and Cmax of phlorizin, and only the Peff value was found to be effective (Fig. 8). In situations where the Tmax and Cmax values simulated using in silico-predicted parameters do not match the actual observed values, as in the case described earlier, our proposal for estimating the Peff value based on the experimental Ka value might be useful.

The TAIC/Tmax of luseogliflozin in rats was 32%, suggesting a low risk of a DDI of luseogliflozin against miglitol absorption. In contrast, phlorizin as a positive control inhibited the absorption of miglitol, since the TAIC/Tmax was more than 100%. To verify the validity of the prediction described earlier, an in vivo interaction study in rats was conducted. Although the plasma concentrations of miglitol until 15 minutes after coadministration of luseogliflozin and miglitol were slightly lower than those after the administration of miglitol alone, no differences in the Cmax and AUC0-t values were observed; thus, luseogliflozin did not inhibit the absorption of miglitol significantly (Fig. 4A). In contrast, phlorizin as a positive control inhibited the absorption of miglitol, reducing the Cmax and AUC0-t of miglitol (Fig. 4B). As described earlier, the validity of the proposed method for predicting DDI risk was proven because the prediction results corresponded to the results of the in vivo interaction study. In addition, the absorption of miglitol was not completely inhibited even in the presence of an excessive concentration of phlorizin. Regarding miglitol absorption, no reports have presented any information other than the fact that miglitol acts as a substrate for SGLT1, so the reason for the result is unclear; however, passive diffusion might contribute to the absorption.

Since the validity of the proposed method was verified in rats, the assessment was subsequently conducted in humans (Figs. 5 and 6).
a result, luseogliflozin was expected to have no DDI potential against miglitol absorption in humans, since the T\textsubscript{AIC/Tmax} (17%) was lower than the value in rats. This expectation was proven in an actual clinical interaction study (Fig. 7).

A DDI study examining ipragliflozin, a novel SGLT2 inhibitor (100 mg/individual, orally), and miglitol (75 mg/individual, orally) has demonstrated that the geometric mean ratios of the C\textsubscript{max} and area under the curve from zero to infinity (AUC\textsubscript{0-\textinfty}) of miglitol for the combination therapy versus monotherapy were 0.761 (90% CI: 0.672–0.861) and 0.796 (90% CI: 0.719–0.881), respectively (http://www.info.pmda.go.jp/downfiles/ph/PDF/800126_3969018F1022_1_01.pdf). Based on this information, we evaluated the DDI potential between luseogliflozin and miglitol retrospectively according to our proposed approach using the published IC\textsubscript{50} value (1876 nM) for hSGLT1 (Tahara et al., 2012) and human PK data for ipragliflozin (Zhang et al., 2013). The resulting T\textsubscript{AIC/Tmax} was estimated to be 116%, predicting that ipragliflozin will interact with miglitol absorption in humans.

The decision criterion for performing a clinical DDI study is an [I2]/IC\textsubscript{50} ≥ 10 according to the FDA draft guidance for DDIs, under an assumption based on the static model that the highest concentration of the inhibitor will persist. Furthermore, a refined criterion of [I2]/IC\textsubscript{50} ≈ 5 under the same assumption has been proposed by Cook et al. (2010). However, in our research, luseogliflozin did not inhibit miglitol absorption at all, even though the [I2]/IC\textsubscript{50} value of luseogliflozin exceeded the cutoff value of 10. This false-positive result was caused by an overestimation as a result of the assumption involved in using a static model. On the other hand, we considered that the evaluation of the actual time-concentration profile of luseogliflozin in the intestine was important to construct an accurate method for predicting the DDI potential. Consequently, the predictions obtained using our proposed method for DDI risk assessment using a dynamic model corresponded to the results of the in vivo interaction study.

In conclusion, a strategy for predicting the DDI potential of a concomitant agent against a drug absorbed via an intestinal transporter was proposed, and luseogliflozin was clearly shown not to cause a DDI against miglitol absorption in humans through a verification of the validity of the proposed prediction method.

The use of this proposed strategy based on the simulation of intestinal concentration-time profiles using dynamic modeling may be of great help in evaluating the clinical DDI potentials of concomitant agents against drugs absorbed via an intestinal transporter without the need to conduct an interaction study.

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Authorship Contributions

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Contributed new reagents or analytic tools: Mizuno-Yasuhira.

Performed data analysis: Mizuno-Yasuhira, Nakai, Gunji, Kinoshita, Jingu, Yamaguchi.

Wrote or contributed to the writing of the manuscript: Mizuno-Yasuhira, Nakai, Takahashi, Kinoshita, Yamaguchi.

References


Mizuno-Yasuhira, Nakai, Uchida, Takahashi, Kinoshita, Yamaguchi.

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