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

Akiko Mizuno-Yasuhira, Yasuhiro Nakai, Emi Gunji, Saeko Uchida, Teisuke Takahashi,
Kohnosuke Kinoshita, Shigeji Jingu, Soichi Sakai, Yoshishige Samukawa, Jun-ichi Yamaguchi

Taisho Pharmaceutical Co., Ltd., Drug Safety and Pharmacokinetics Laboratories, Saitama, Japan (A.M., K.K., S.J., J.Y.); Development Headquarters, Tokyo, Japan (Y.N., Y.S.); Molecular Function and Pharmacology Laboratories, Saitama, Japan (E.G., S.U., T.T.); and Clinical Research, Tokyo, Japan (S.S.)
Running Title

Prediction of transporter-mediated DDI using a dynamic model

Corresponding Author

Akiko Mizuno-Yasuhira

1-403, Yoshino-cho, Kita-ku, Saitama-shi, Saitama 331-9530, Japan

TEL: +81-48-669-3036

FAX: +81-48-652-7254

E-mail: a-yasuhira@so.taisho.co.jp

Number of text pages: 41

Number of tables: 4

Number of figures: 8

Number of references: 28

Number of words in the abstract: 249

Number of words in the introduction: 750

Number of words in the discussion: 1301
Abbreviations

AIC, Akaike information criterion; AUC, area under the curve; CIs, confidence intervals; CL, total clearance;

C_{max}, maximum concentration; DDI, drug-drug interaction; Fa, fraction absorbed value; FDA, U.S. Food and

Drug Administration; Fg, intestinal availability; Fh, hepatic availability; FPE, first-pass effect; IC_{50}, 50%
inhibitory concentration; IS, internal standard; [I_2], concentration of inhibitor in the gastrointestinal tract arising

from the highest approved clinical dose dissolved in 250 mL of water; Ka, absorption rate constant; k_{12}, rate

constant for compartment 1 to 2; k_{21}, rate constant for compartment 2 to 1; LC-MS/MS, liquid

chromatography-tandem mass spectrometry; \alpha-MG, methyl-\alpha-D-glucopyranoside; P_{eff}, effective permeability;

PK, pharmacokinetics; SGLT, sodium-glucose cotransporter; TAIC, time spent above a value 10-fold higher

than the IC_{50} value; T_{max}, time for C_{max}; Vc, volume of distribution
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 antidiabetic drug, against miglitol absorbed via the intestinal sodium-glucose cotransporter 1 (SGLT1). The method involves four steps: [step1], collection of physicochemical and pharmacokinetic parameters of luseogliflozin for use in a computer simulation; [step2], evaluation of the validity of these parameters by verifying the goodness-of-fit between simulated and observed plasma profiles; [step3], simulation of the intestinal luseogliflozin concentration-time profile using the ACAT model in GastroPlus™ and estimation the time spent above a value 10-fold higher than the IC_{50} value (TAIC) for SGLT1; and [step4], the evaluation of the DDI potential of luseogliflozin by considering the percentage of TAIC against the miglitol T_{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 the 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 (Fahmi et al., 2009; Kato et al., 2008). 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 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.

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; Hummel et al., 2010; Chen et al., 2010). In the current study, miglitol and luseogliflozin were adopted as model compounds for an 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 alpha-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 et al., 2013) that is used orally 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; 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 non-selective 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 $[I_2]$, which 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 ($IC_{50}$) value for SGLT1 ($SGLT IC_{50}$). The value of $[I_2]/SGLT IC_{50}$ was higher than 10, which is the value used as a decision criteria 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 luseogliiflozin against miglitol.
Materials and Methods

Materials

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 isotope-labeled luseogliflozin (\(^2\text{H}_5\)-luseogliflozin), which was used as an internal standard (IS) for the quantitative analysis of both luseogliflozin and phlorizin, and stable isotope-labeled miglitol (\(^2\text{H}_4\)-miglitol), which was used as an IS for the quantitative analysis of miglitol were synthesized at Taisho Pharmaceutical Co., Ltd. (Saitama, Japan). The chemical structures of luseogliflozin, miglitol, and phlorizin are shown in Fig. 1. Methyl-\(\alpha\)-D-glucopyranoside (\(\alpha\)-MG) and \([^{14}\text{C}]\ \alpha\)-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, Inc.) were used for the experiments. All 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).
**Determination of IC$_{50}$ Value for SGLT1**

Chinese hamster ovary -K1 cells (CHO-K1) were obtained from the American Type Culture Collection (Rockville, MD) and were stably transfected with a plasmid vector for human (h) SGLT1 (Genbank Accession number, NM_000343). The cells were cultured in a 96-well plate in F-10 Nutrient Mixture containing 10% fetal bovine serum and 250 μg/mL of hygromycin for 2 days. African green monkey SV40-transfected kidney fibroblast cells (COS-7) obtained from the American Type Culture Collection (Rockville, MD) were seeded into a 96-well plate in Dulbecco’s Modified Eagle’s Medium (DMEM) 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 (r) SGLT1 (Genbank Accession number, NM_013033) and cultured for 1 day. The cells expressing rSGLT1 or hSGLT1 were incubated in a pre-treatment 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 min. 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 α-MG substrate mixture ([1$^{14}$C]-α-MG and α-MG) and various concentrations of the test agents or the vehicle alone (DMSO) at 37˚C for 20 or 30 min for the rSGLT1 or hSGLT1 assay, which were each performed in triplicate. The concentration of the α-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 pre-treatment buffer containing 10 mM α-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 CaCl₂, 1 mM MgCl₂, 10 mM HEPES, 5 mM Tris, pH 7.2–7.4) containing an α-MG substrate mixture ([¹⁴C]-α-MG and α-MG, 500 μM for rSGLT1, 1 mM for hSGLT1) and DMSO as the vehicle. The sodium-dependent α-MG uptake was calculated by subtracting of the sodium-independent uptake count from each count measured in the uptake buffer [Na+]. The IC₅₀ values were determined using SAS 8.2 (SAS Institute Inc., Cary, NC).

**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™ Ver. 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 the GastroPlus™ is the 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 (TAIC) in the intestine was estimated for both luseogliflozin and phlorizin using the procedure described below. The parameters for the computer simulation are shown in Table 1.

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 of phlorizin in rat was estimated in the same way.

The effective permeability (Peff) values were estimated using the following equation (Amidon et al., 1995):

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

(1),

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 (Komiyama et al., 1980) and 1.75 cm for humans (Fagerholm et al., 1996).

2) Estimation of fraction absorbed value and PK parameters
The fraction absorbed value (Fa) was predicted by importing 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 h based on the recommended value, since the dosage form was not a tablet, but a solution or suspension. The PK parameters (CL, Vc, k12, k21) of luseogliflozin and phlorizin in rat were obtained by fitting the i.v. plasma concentration-time data using the PKPlus™ module, and the parameters (CL/F, Vc/F, k12, k21) of luseogliflozin in humans were obtained by fitting the p.o. plasma concentration-time data 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).

3) Estimation of hepatic and intestinal first-pass effect

The hepatic and intestinal first-pass effects (FPEs) were estimated as follows:

FPE Liver(%): \((1 - \text{Fh}) \times 100\), and

FPE Intestinal(%): \((1 - \text{Fg}) \times 100\),

where Fh is the hepatic availability and Fg is the intestinal availability. The hepatic clearance was assumed to be equal to the total clearance (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:
\[ F_h = 1 - \frac{CL}{R_b} \frac{Q_h}{Q_b} \] (2), and

\[ F_g = \frac{F}{Fa \times F_h} \] (3),

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.

4) 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/P200500031/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 Rhoads, 1994) but no further data was 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, while 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 3% isoflurane (Mylan Inc., Canonsburg, PA) and a total O₂ flow rate of 0.5–1 L/min using an RC2 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 min and at 1, 2, 4, 8, and 24 h 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 (Japanese healthy 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 luseogliflozin. Blood samples obtained
during both studies were collected at pre, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h 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_{max}**

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

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

**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 an SPE cartridge (Mono Spin C18-CX, GL Science, Tokyo, Japan). Miglitol and the IS were eluted by centrifugation at preset values: 10600 xg, 4°C, 1 min. A 5-µL
aliquot of the eluate was then injected into the LC-MS/MS system (Mizuno-Yasuhiro et al., 2014).

Luseogliflozin and Phlorizin in Rat Plasma

Twenty-five microliters of IS solution (100 ng/mL) and 500 µL of 10 mmol/L 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 min). The eluate was evaporated to dryness and dissolved in 100 µL of acetonitrile/10 mmol/L 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 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 r/min, 5 min) and centrifuged (preset values: 400×g, 4°C, 5 min). 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. Miglitol was analyzed using an XBridge Amide column (4.6 mm I.D. × 50 mm, 3.5-µm particle size; Waters, Milford, MA) with 10 mmol/L of 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, 5-µm particle size; GL Science, Tokyo, Japan) with 1 mmol/L of ammonium acetate and acetonitrile as the mobile phase under a gradient condition. The selected reaction monitoring (SRM) 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, Milford, MA) and API4000 mass spectrometer (AB SCIEX, Foster City, CA). 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
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 (C<sub>max</sub> and AUC<sub>0-t</sub>) between miglitol alone and the combination groups. The ratio of the geometric mean and its 90% CI were estimated using an analysis of variance (ANOVA) model with treatment as a fixed effect and with the subject as a random effect using a SAS mixed procedure (ver.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 IC<sub>50</sub> 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 IC<sub>50</sub> of 895 nM but was less potent for
hSGLT1 (IC$_{50}$ value: 2900 nM). Phlorizin also inhibited sodium-dependent α-MG uptake with an IC$_{50}$ 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 utilized in the GastroPlus™ computer simulation are summarized in Table 1. The $P_{eff}$ values of luseogliflozin and phlorizin in rats estimated from each $K_a$ value were 6.23 cm/s x $10^4$ and 0.558 cm/s x $10^4$, respectively. The values of the other physicochemical parameters were estimated using ADMET Predictor™ or were observed. The PK parameters ($CL$, $V_c$, $k_{12}$, $k_{21}$) 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, 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, 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 C_{max} and AUC_{0-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 above, 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 IC_{50} value for SGLT1, as shown in Fig. 3. The estimated TAIC values of luseogliflozin and phlorizin in rats were 9 min and 75 min, respectively.

**Prediction of the DDI Potential in Rats by Considering the Percentage of TAIC Against the Miglitol T_{max}**

**Value (TAIC/T_{max})**

The T_{max} of miglitol after oral administration to rats was 28 min, 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. 4 (A)). Luseogliflozin had no effect on the miglitol PK parameters (C\text{max}, AUC\text{0-t}), 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 h after the co-administration of miglitol and phlorizin were lower than after the administration of miglitol alone (Fig. 4 (B)). The 90% CI for the ratios of the C\text{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\text{0-t} 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 x 10^4. 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. 5 (A-1)), which produced the best fit for the data as assessed
using the AIC. Then, the plasma concentration-time profile of luseogliflozin (5 mg/man) was simulated using the ACAT model in GastroPlus™. As in the case for rats, as shown in Fig. 5 (A-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 IC₅₀ value for SGLT1, as shown in Fig. 6. The estimated TAIC of luseogliflozin in humans was 14 min.

**Prediction of the DDI Potential in Humans by Considering the Percentage of TAIC Against the Miglitol Tmax Value (TAIC/Tmax)**

The Tmax of miglitol after oral administration in humans was 83 min (Fig. 7). The TAIC/Tmax of luseogliflozin against miglitol absorption in humans was 17%. The TAIC/Tmax 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/man) in combination with luseogliflozin (5 mg/man) 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.

**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/T\textsubscript{max}, was considered to be important for accurate assessments. The T\textsubscript{max} is easy to monitor and was set as the absorption duration of a drug, while 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 absorbed via intestinal SGLT1.

The proposed prediction method can be summarized in four steps: [step1], the collection of physicochemical and PK parameters of luseogliflozin for use in a computer simulation; [step2], the evaluation of the validity of these parameters by verifying the of goodness-of-fit between simulated and observed plasma profiles; [step3], the simulation of the intestinal luseogliflozin concentration-time profile using the ACAT model in GastroPlus\textsuperscript{TM} and estimation the TAIC ; and [step4], the evaluation of the DDI potential by considering the TAIC/T\textsubscript{max}.
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 [step2] when the simulation was conducted using the $P_{eff}$ value predicted by the built-in ADMET Predictor™ at [step1]. The absorption velocity of the simulated concentration-time profiles seemed to be lower than the observed concentration as the results of the low $P_{eff}$ value. The observed $T_{max}$ values of luseogliflozin and phlorizin were 0.25 h, but the simulated $T_{max}$ values were 0.64 h and 1.27 h, respectively. Furthermore, the simulated $C_{max}$ value of phlorizin also deviated from that of the observed data. Therefore, referring to a report
by Amidon et al. (1995), the P_{eff} values in rats were estimated based on each experimental Ka value using equation (1). Using these P_{eff} values, the simulated concentration-time profiles and the observed values were well matched, and the T_{max} values of luseogliflozin and phlorizin improved to 0.37 h and 0.51 h, respectively (Fig. 2 (A-2, B-2)). Under these conditions, the PK of luseogliflozin and of phlorizin in rats were 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 T_{max} of luseogliflozin and the T_{max} and C_{max} of phlorizin, and only the P_{eff} value was found to be effective (Fig. 8). In situation where the T_{max} and C_{max} values simulated using in silico-predicted parameters do not match the actual observed values, as in the case described above, our proposal for estimating the P_{eff} value based on the experimental Ka value might be useful.

The TAIC/T_{max} 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/T_{max} was more than 100%. To verify the validity of the prediction described above, an in vivo interaction study in rats was conducted. Although the plasma concentrations of miglitol until 15 min after co-administration of luseogliflozin and miglitol were slightly lower than those after the administration of miglitol alone, no differences in the C_{max} and AUC_{0-1} values were observed; thus, luseogliflozin did not inhibit the absorption of miglitol significantly (Fig. 4 (A)). In contrast, phlorizin as a positive control inhibited the absorption of miglitol, reducing the C_{max} and AUC of miglitol (Fig. 4 (B)). As described above, 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 (Fig. 5, 6). As a result, luseogliflozin was expected to have no DDI potential against miglitol absorption in humans, since the TAIC/T_{max} (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/man, orally), and miglitol (75 mg/man, orally) has demonstrated that the geometric mean ratio of the C_{max} and AUC_{inf} 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 ipragliflozin 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 TAIC/T_{max} was estimated to be 116%, predicting that ipragliflozin may interact with miglitol absorption in humans.
The decision criterion for performing a clinical DDI study is an \([I_2]/IC_{50} \geq 10\) 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 \([I_2]/IC_{50} \geq 5\) under the same assumption has been proposed by Cook et al. (2009). However, in our research, luseogliflozin did not inhibit miglitol absorption at all, even though the \([I_2]/IC_{50}\) 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.
Acknowledgments

We thank Ms. Yoko Mano for her contributions to the GastroPlus™ simulations and Mr. Yasunori Kawakita for his bioanalytic support of the studies.

Authorship Contribution

Participated in research design: Mizuno-Yasuhira, Nakai, Uchida, Takahashi, Kinoshita, Jingu, Sakai, Samukawa, Yamaguchi

Conducted experiments: Mizuno-Yasuhira, Gunji

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


Drug Metab dispos 37: 1658–1666.


(1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (TS-071) is a potent, selective sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor for type 2 diabetes treatment. J Med Chem 53: 3247–3261.


Schmiedebergs Arch Pharmacol 385: 423–436.


**Figure Legends**

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

Fig.2. Plasma concentration time profiles for luseogliflozin and phlorizin in rats.

A-1 and B-1), Two-compartment model-fitted profiles in rats after the single intravenous administration of (A-1) luseogliflozin (0.1 mg/kg) and (B-1) phlorizin (4 mg/kg). The closed circles represent the mean observed data + S.D. (n = 3). The solid lines were fitted using a nonlinear least-squares regression analysis. The PK parameters (CL, Vc, k_{12}, k_{21}) in rats for simulating luminal concentrations were obtained by this fitting to the observed data.

A-2 and B-2), Simulated concentration-time profiles in rats after the single oral administration of (A-2) luseogliflozin (0.1 mg/kg) and (B-2) phlorizin (40 mg/kg). The closed circles represent the mean observed data + S.D. (n = 6). The solid lines represent the model-simulated profiles, which were obtained using the ACAT model in GastroPlus™.

Fig.3. Simulated luminal concentration-time profiles in rat duodenum (red line) and time spent above a value 10-fold higher than the IC_{50} value (TAIC) after the single oral administration of (A) luseogliflozin (0.1 mg/kg) and (B) phlorizin (40 mg/kg).
The dashed lines represent a value 10-fold higher than the IC$_{50}$ value for SGLT1. The TAIC of luseogliflozin in rats (9 min) was shorter than the T$_{max}$ of miglitol in rats (28 min). On the other hand, the TAIC of phlorizin in rats (75 min) was longer.

Fig. 4. Plasma levels of miglitol in rats following the oral administration of miglitol alone (1.5 mg/kg, red line) or in combination with concomitant agents.

A, Combination with luseogliflozin (0.1 mg/kg, blue line); B, combination with phlorizin (40 mg/kg, green line). The closed circles and triangles represent the observed mean data ± S.D. (n = 6). The data for miglitol alone was referenced from Mizuno-Yasuhira et al. (2014).

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/man). The solid line was fitted using a nonlinear least-squares regression analysis. The PK parameters (CL/F, Vc/F, k$_{12}$, k$_{21}$) in humans for simulating luminal concentrations were 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/man). The solid lines represent the model-simulated profiles, which were obtained using the ACAT
model in GastroPlus™.

Fig. 6. Simulated luminal concentration-time profiles in human duodenum (red line), upper jejunum (green
line), and time spent above a value 10-fold higher than the IC₅₀ value (TAIC) after the single oral
administration of luseogliflozin (5 mg/man).

The dashed line represents a value 10-fold higher than the IC₅₀ value for SGLT1. The TAIC of luseogliflozin
in humans (14 min) was shorter than the Tₘₐₓ of miglitol in humans (83 min).

Fig. 7. Plasma levels of miglitol in humans following the oral administration of miglitol alone (50 mg/man,
red line) or in combination with luseogliflozin (5 mg/man, blue line).

The closed circles and triangles represent the observed mean data ± S.D. (n = 12).

Fig. 8. Potential influence of Pₑffect value on the Tₘₐₓ and Cₘₐₓ values.

The Tₘₐₓ of luseogliflozin and the Tₘₐₓ and Cₘₐₓ of phlorizin were simulated using different values for Pₑffect
with other parameters fixed at the same values, as shown in Table 1.
Table 1  Input parameters for GastroPlus™ that were used to simulate the luminal concentrations

<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>Molecular Formula</td>
<td>C₂₅H₃₀O₆S</td>
<td>-</td>
<td>C₂₁H₂₄O₁₀</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 @pH=6.57)</td>
<td>0.0771</td>
<td>In-house data</td>
<td>9.27</td>
</tr>
<tr>
<td>Diff. Coeff. (cm²/s x 10⁵)</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>Peff (cm²/s x 10⁵)</td>
<td>6.23</td>
<td>Estimated from Ka</td>
<td>0.558</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>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>Use Exp Plasma 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>-</td>
<td>2-compartment</td>
</tr>
<tr>
<td>CL (L/h)</td>
<td>0.495</td>
<td>-</td>
<td>0.766</td>
</tr>
<tr>
<td>Vc (L/kg)</td>
<td>1.21</td>
<td>Fitted from 0.1 mg/kg i.v. data</td>
<td>0.877</td>
</tr>
<tr>
<td>k₁₂ (1/h)</td>
<td>0.384</td>
<td>-</td>
<td>0.0352</td>
</tr>
<tr>
<td>k₂₁ (1/h)</td>
<td>0.271</td>
<td>-</td>
<td>0.535</td>
</tr>
</tbody>
</table>

Peff, effective permeability; ASF, absorption scaling factor; FPE, first-pass effect; CL, clearance; Vc, volume of distribution; k₁₂, rate constant for compartments 1 to 2; k₂₁, rate constant for compartments 2 to 1; IR, immediate release; Ka, absorption rate constant; F, bioavailability.
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><strong>Pharmacokinetics</strong></td>
<td>Luseogliflozin</td>
<td>iv</td>
<td>3</td>
<td>0.1</td>
<td>0.1</td>
<td>10% HP-β-CD solution</td>
<td></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*</td>
<td>0.5% CMC-Na solution</td>
<td></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 suspension</td>
<td></td>
</tr>
<tr>
<td><strong>Interaction</strong></td>
<td>Miglitol alone</td>
<td>po</td>
<td>6</td>
<td>1.5</td>
<td>0.225*</td>
<td>0.5% CMC-Na solution</td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>Miglitol</td>
<td>po</td>
<td>6</td>
<td>0.1</td>
<td>0.225*</td>
<td>0.5% CMC-Na solution</td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>Luseogliflozin</td>
<td>po</td>
<td>6</td>
<td>1.5</td>
<td>0.225*</td>
<td>0.5% CMC-Na suspension</td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>Miglitol</td>
<td>po</td>
<td>6</td>
<td>1.5</td>
<td>0.225*</td>
<td>0.5% CMC-Na suspension</td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>Phlorizin</td>
<td>po</td>
<td>6</td>
<td>40</td>
<td>6</td>
<td>0.5% CMC-Na suspension</td>
<td></td>
</tr>
</tbody>
</table>

N, number of animals; 10% HP-β-CD, 10% hydroxy propylβ cyclodextrin; PEG400, polyethylene glycol 400;
CMC-Na, carboxy methyl cellulose sodium.

*The concentration values were set based on the clinical dose regimens.

bReferred from Mizuno-Yasuhira et al., 2014.
Table 3  Related parameters for the prediction of drug-drug interaction potential

<table>
<thead>
<tr>
<th></th>
<th>Luseogliflozin in rats</th>
<th>Phlorizin in rats</th>
<th>Luseogliflozin in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-fold of SGLT1 IC₅₀ (µg/mL)</td>
<td>3.89</td>
<td>2.75</td>
<td>12.6</td>
</tr>
<tr>
<td>Dose</td>
<td>0.1 mg/kg</td>
<td>40 mg/kg</td>
<td>5 mg/man</td>
</tr>
<tr>
<td>𝑇ₘₐₓ of miglitol (min)</td>
<td>28ᵃ</td>
<td>28ᵃ</td>
<td>83</td>
</tr>
<tr>
<td>TAIC (min)</td>
<td>9</td>
<td>75</td>
<td>14</td>
</tr>
<tr>
<td>TAIC/𝑇ₘₐₓ (%)</td>
<td>32</td>
<td>268</td>
<td>17</td>
</tr>
</tbody>
</table>

TAIC, time spent above a value 10-fold higher than the IC₅₀ value; also shown in Figure 3 and Figure 6.

ᵃReferenced from Mizuno-Yasuhiro et al., 2014.
Table 4  Summary of statistical analysis of miglitol pharmacokinetics parameters in rats

<table>
<thead>
<tr>
<th>Combination</th>
<th>Parameter</th>
<th>Point estimate</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(combination/miglitol alone)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Miglitol and luseogliflozin</td>
<td>$C_{\text{max}}$</td>
<td>0.97</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>$AUC_{0-t}$</td>
<td>1.12</td>
<td>1.04</td>
</tr>
<tr>
<td>Miglitol and phlorizin</td>
<td>$C_{\text{max}}$</td>
<td>0.41*</td>
<td>0.23*</td>
</tr>
<tr>
<td></td>
<td>$AUC_{0-t}$</td>
<td>0.63*</td>
<td>0.38*</td>
</tr>
</tbody>
</table>

90% CI, 90% confidence interval.

*The 90% CIs of the ratio (combination/miglitol alone) was outside the range of 0.8–1.25.
Fig. 1
Fig. 2
Fig. 3
Fig. 4

A  
- Red line: Miglitol alone
- Blue line: Combination with luseogliflozin

B  
- Red line: Miglitol alone
- Green line: Combination with phlorizin
Fig. 5

Plasma concentration of luseogliflozin (ng/mL) vs. time (h) for two different conditions labeled A-1 and A-2.
Time (h)
Luminal concentration of luseogliflozin (μg/mL)
10-fold of IC₅₀ value
12.6 μg/mL
TAIC: 14 min

14 min << 83 min (Tₘₐₓ of miglitol in humans)

Fig. 6
Fig. 7
Fig. 8