In Vitro–In Vivo Correlation of the Inhibition Potency of Sodium-Glucose Cotransporter Inhibitors in Rat: A Pharmacokinetic and Pharmacodynamic Modeling Approach

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Received January 8, 2013; accepted February 4, 2013

ABSTRACT

To evaluate the relationship between the in vitro and in vivo potency of sodium-glucose cotransporter (SGLT) inhibitors, a pharmacokinetic and pharmacodynamic (PK-PD) study was performed using normal rats. A highly selective SGLT2 inhibitor, tofogliflozin, and four other inhibitors with different in vitro inhibition potency to SGLT2 and selectivity toward SGLT2, versus SGLT1 were used as test compounds, and the time courses for urinary glucose excretion (UGE) and the plasma glucose and compound concentrations were monitored after administration of the compounds. A PK-PD analysis of the UGE caused by SGLT inhibition was performed on the basis of a nonlinear parallel tube model that took into consideration the consecutive reabsorption by different glucose transport properties of SGLT2 and SGLT1. The model adequately captured the time course of cumulative UGE caused by SGLT inhibition; then, the in vivo inhibition constants (Ki) of inhibitors for both SGLT1 and SGLT2 were estimated. The in vivo selectivity toward SGLT2 showed a good correlation with the in vitro data (r = 0.985; P < 0.05), with in vivo Ki values for SGLT2 in the range of 0.3–3.4-fold the in vitro data. This suggests that in vitro inhibition potency to both SGLT2 and SGLT1 is reflected in vivo. Furthermore, the complementary role of SGLT1 to SGLT2 and how selectivity toward SGLT2 affects the inhibitory potency for renal glucose reabsorption were discussed using the PK-PD model.

Introduction

In normal animals and subjects, glucose is filtered at the glomerulus of the kidney and almost completely reabsorbed by sodium-glucose cotransporters (SGLT) expressed in the proximal tubules. Two isoforms of SGLT with different affinity to glucose are localized at different positions in the proximal tubule: a low-affinity and high-capacity transporter (SGLT2) is expressed in the convoluted tubule, and a high-affinity and low-capacity transporter (SGLT1) is expressed in the straight tubule (Turner and Moran, 1982; Hediger and Rhoads, 1994; Oulianova and Berteloot, 1996; Wright, 2001). The glucosuria observed in SGLT2 knockout mice (Jureczak et al., 2011; Vallon et al., 2011) and in familial patients with renal glucosuria (Elsas and Rosenberg, 1969) with SGLT2 mutation (Magen et al., 2005) indicates that SGLT2 plays a predominant role in renal glucose reabsorption in the kidney.

It has been reported that inhibiting SGLT increases urinary glucose excretion (UGE) and reduces blood glucose level, which improves glycemic control in an insulin-independent manner (Rossetti et al., 1987; Oku et al., 2000). Accordingly, several SGLT inhibitors are currently under clinical development for type II diabetes treatment (Abdul-Ghani et al., 2011; Kipnes, 2011). Considering that two isoforms of SGLT with different properties contribute to renal glucose reabsorption and that SGLT1 is expressed in not only the kidney but also the small intestine (Hediger and Rhoads, 1994), the inhibition potency to each transporter would be an important factor in efficacy and safety. Although both SGLT1 and SGLT2 in the kidney can be targets for type II diabetes treatment, most SGLT inhibitors being developed have high selectivity toward SGLT2 versus SGLT1 to avoid some possible adverse events that may be caused by SGLT1 inhibition (Jabbour and Goldstein, 2008). SGLT2 inhibitors are known to exhibit different levels of glucosuric potency (List and Whaley, 2011), and both the in vitro SGLT2
inhibition constant and selectivity toward SGLT2 would be important factors in the in vivo inhibitory effect on renal glucose reabsorption. Moreover, pharmacokinetic properties, such as bioavailability and half-life, would also be a considerable issue for in vivo effectiveness. As described above, many factors affect the in vivo efficacy of an SGLT inhibitor, making it difficult to predict in vivo efficacy from in vitro data or, in this specific case, to know how in vitro inhibition potency to SGLT2 and selectivity toward SGLT2 would be reflected in vivo.

The purpose of this study was to evaluate the in vitro–in vivo relationship of the inhibition constant ($K_i$) of SGLT inhibitors. Tofogliflozin is a highly potent and selective SGLT2 inhibitor (Ohtake et al., 2012; Suzuki et al., 2012) and is currently in a phase III trial for type II diabetes mellitus treatment. We used tofogliflozin and other inhibitors with different in vitro inhibition potency to SGLT2 and selectivity toward SGLT2 as the test compounds and performed pharmacokinetic and pharmacodynamic (PK-PD) studies of them with use of normal rats. We previously developed a mechanism-based PK-PD model in which the different glucose transport properties between SGLT1 and SGLT2 were considered (Yamaguchi et al., 2011). The model was able to describe the plasma glucose-dependent UGE in rats; moreover, it could estimate the in vivo inhibition constants of SGLT1 and SGLT2 for phlorizin, which has less selectivity toward SGLT2. In the present study, we used the PK-PD model to estimate the in vivo $K_i$ of SGLT inhibitors by analyzing the relationship between UGE and plasma inhibitor concentration and then analyzed the in vitro–in vivo relationship for $K_i$. Furthermore, a simulation study was performed to understand how selectivity toward SGLT2 affects the inhibitory effect on renal glucose reabsorption under euglycemic and hyperglycemic conditions.

**Materials and Methods**

**Chemicals.** (1S, 3'R, 4'S, 5'S, 6'R)-6-[(4-ethylphenyl)methyl]-3',4,5,6-tetrahydro-6'-(hydroxymethyl)-spirol[isobenzofuran-1(3'H), 2',2'H] pyran]-3',4,5-triol (tofogliflozin) (Ohtake et al., 2012), 2-[4-methoxyphenyl]methyl]phenyl]hex-6-0-ethoxycarbonyl-6-D-glucopyranoside (sergliflozin) (Katsumo et al., 2007), 2-[4-methoxyphenyl]methyl]phenyl]-6-D-glucopyranoside (sergliflozin-A, an active form of sergliflozin), 3-[benz[b]furan-5-yl]-6,8-dihydroxy-4'-methylpropiope none-2-O-6-O- methoxycarbonyl]-6-D-glucopyranoside (T-1095) (Oku et al., 1999), 3-[benz[b]furan-5-yl]-2,6'-dihydroxy-4'-methylpropiope none-2'-O-6-D-glucopyranoside (T-1095A, an active form of T-1095) (Oku et al., 1999), and (2S,3R,4'R,5'R,6'R)-2-[(4-ethylbenzyl)-phenyl]-6-hydroxymethyl-tetrahydro- 2H- pyran-3,4,5-triol (BMS, T-1095) (Oku et al., 1999), 3-(benz[b]furan-5-yl)-2,6'-dihydroxy-4'-methylpropiope none-2'-O-6-D-glucopyranoside (T-1095A, an active form of T-1095) (Oku et al., 1999), and (2S,3R,4'R,5'R,6'R)-2-[(4-ethylbenzyl)- (phenyl)-6-hydroxymethyl-tetrahydro- 2Hpyran-3,4,5-triol (BMS, a patent compound of Bristol-Myers Squibb Pharmaceutical Co., Ltd., New York, NY) (Ellsworth et al., 2001; Deshpande et al., 2012) were synthesized in Chugai Pharmaceutical Co., Ltd. and were approved by the Ethics Committee for Treatment of the animals and the protocols were performed in accordance with the Guidelines for the Care and Use of Animals at Chugai Pharmaceutical Co., Ltd. and were approved by the Ethics Committee for Treatment of Laboratory Animals at Chugai Pharmaceuticals.

**Inhibition Study of AMG Uptake Using Cells Expressing Rat and Mouse SGLTs.** In vitro inhibition studies using cells expressing rat SGLT1 (rSGLT1), rat SGLT2 (rSGLT2), mouse SGLT1 (mSGLT1), and mouse SGLT2 (mSGLT2) were performed by the method previously reported (Suzuki et al., 2012). Rat and mouse SGLTs cDNA was amplified with total RNA isolated from the kidney or small intestine of a Wistar rat or a db/db mouse. Polymerase chain reaction (PCR) primers were designed from published sequences [GenBank accession number: NM001107229 (rSGLT1), NM022590 (rSGLT2), BC0383845 (mSGLT1), AY033886 (mSGLT2)]. The sequences of PCR primers used were ACCCTCGAAGACTCAAGATATAAGG and ACCCATATCATCATCTTTTATCCGAATGAG for rSGLT1, AACCTAAACACGATAAGAG and ACATGCCTCGGTGCAACT for rSGLT2, ACCCTCGAGAAGCTGACATGCATAAGG and ACCCATATGAGGGCTCAAGGAACAGTAGGAC and ACCGAATCCACCCCTGACTTTGTAGCAT for mSGLT1, and ACCCTGGAGAGGACACAGTAGGACCCGACAGATAGGAC for mSGLT2. Experimental conditions for PCR with KOD Plus (TOYOBO Co., Osaka, Japan) were as follows: 94°C for 2 minutes and 35 cycles of 94°C for 15 seconds, 58°C for 30 seconds, and 68°C for 3 minutes. Expression plasmids containing rat and mouse SGLTs were prepared using ligating amplified cDNA fragments into the multiloning site of pcDNA3.1(+) [Life Technologies Co. (Invitrogen), Grand Island, NY]. The expression plasmids containing mSGLT1 and mSGLT2 cDNA fragment were transfected into Chinese hamster ovary-K1 cells (American Type Culture Collection, Manassas, VA). Clones stably expressing each SGLT were used for the AMG uptake assay. The expression plasmids containing rSGLT1 and rSGLT2 cDNA fragments were transfected into African

Fig. 1. Chemical structures of SGLT inhibitors: tofogliflozin (A), BMS (B), sergliflozin (C), sergliflozin-A (D), T-1095 (E), and T-1095A (F).
green monkey SV40-transfected kidney fibroblast cells (COS-7, American Type Culture Collection), and the cells transiently expressing each SGLT were used for the AMG uptake assay.

For the AMG uptake assay, the cells expressing each SGLT were cultured in 96-well plates for 2 or 3 days and washed twice with sodium-free buffer [140 mM choline chloride, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES/Tris (pH 7.4)]. The cells were then incubated in sodium-free buffer or sodium buffer [140 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES/Tris (pH 7.4)] each containing 1mM AMG mixture (nonradioiodinated AMG and [¹⁴C]-AMG) at 37°C for 45 minutes. Sodium-dependent AMG uptake was calculated by subtracting the radioactivity detected in cells incubated in the sodium-free buffer from the radioactivity detected in the cells incubated in the sodium buffer. IC₅₀ values of SGLT inhibitors were calculated using the empirical four-parameter model fitting of XLfit (IBDS, Guildford, UK) and were indicated as mean values of 2–4 independent experiments. Selectivity toward SGLT2 versus SGLT1 was calculated by dividing IC₅₀ for SGLT1 by that for SGLT2.

Rat Serum Protein Binding Assay. Rat serum protein binding of each test compound was measured using the equilibrium dialysis method; 100 mM potassium phosphate–buffered solution (pH 7.4) was added to a receptor chamber, and rat serum containing a test compound (final concentration: 100 ng/ml) was added to a donor chamber. The equilibrium dialysis apparatus was incubated in a shaking bath for 24 hours at 37°C. After incubation, buffer and serum samples were collected, and the test compound concentrations in serum samples were determined using a high-performance liquid chromatography (HPLC) sample. Standard samples for calibration were prepared using the same method with standard solutions instead of blank serum. Phenyltoin (500 ng/ml) was used as the internal standard for tofogliflozin, BMS, and sergliflozin-A, and indomethacin (200 ng/ml) was used as the internal standard for T-1095A.

The HPLC sample was injected at a volume of 20 µl, and the drug concentration was measured using the following LC-MS/MS method. The LC-MS/MS analysis was performed by coupling a liquid chromatography system to an API300 mass spectrometer (Applied Biosystems/MDS SCIEX, Concord, ON, Canada). The HPLC apparatus consisted of a pump (LC-10AD; Shimadzu Co., Kyoto, Japan) and an auto injector (SIL-HTC; Shimadzu Co.). A CAPCELL PAK C18 column (150 mm; 5 µm) was used as the analytical column. The mobile phase was methanol/10 mM ammonium acetate (4:6, v/v) at a flow rate of 0.2 ml/min. The HPLC eluate was introduced into the source with use of a Turbolon-Spray interface (Applied Biosystems/MDS SCIEX), and the mass spectrometer was operated in positive ion mode for tofogliflozin, BMS, and sergliflozin-A and in negative ion mode for T-1095A. Selected ions were m/z 449.0 for tofogliflozin, m/z 119.1 for BMS, m/z 180.1 (daughter ion of 180.1) for sergliflozin-A, m/z 295.0 (daughter ion of 295.0) for T-1095A, m/z 181.2 (daughter ion of 181.2) for phenyltoin, and m/z 312.0 (daughter ion of 312.0) for indomethacin. Run cycles of measurements were set at 10 minutes (tofogliflozin and BMS), 6 minutes (T-1095A), and 13 minutes (sergliflozin-A). The linear calibration curve for each compound using peak area was obtained in the range of 10–2,000 ng/ml for tofogliflozin, 1–1,000 ng/ml for BMS, 30–3,000 ng/ml for sergliflozin-A, and 3–300 ng/ml for T-1095A, with weighting 1/y.

Estimation of Renal Clearance and Mean Plasma Concentration. The renal glucose clearance ([CLR]Glù) during each urine sampling period was estimated using the following equation:

\[ CLR_{Glù} = \frac{\text{C}_{Ur,Glù} \times V}{AUC_{Glù}} \]

where \( \text{C}_{Ur,Glù} \) is the concentration of glucose in urine, \( V \) is the urine volume, and \( AUC_{Glù} \) is the area under the time–plasma concentration curve for glucose during the corresponding urine-sampling period (\( t \)).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Administration route</th>
<th>Vehicle</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tofogliflozin</td>
<td>Intravenous</td>
<td>PEG400/saline (4.6, v/v)</td>
<td>3</td>
</tr>
<tr>
<td>BMS</td>
<td>Oral</td>
<td>PEG400/saline (4.6, v/v)</td>
<td>10</td>
</tr>
<tr>
<td>Sergliflozin</td>
<td>Intravenous</td>
<td>PEG400/saline (1.1, v/v)</td>
<td>10</td>
</tr>
<tr>
<td>T-1095</td>
<td>Intravenous</td>
<td>PEG400/saline (1.1, v/v)</td>
<td>400</td>
</tr>
</tbody>
</table>

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The mean plasma concentrations of glucose ($C_{AV,Glc}$) and test compound ($C_{AV,Drug}$) were also estimated using the following equations:

$$C_{AV,Glc} = \frac{AUC_{Glc}}{\tau}, \quad C_{AV,Drug} = \frac{AUC_{Drug}}{\tau}$$  \hspace{2cm} (3)

where $AUC_{Drug}$ is the area under the time-plasma concentration curve for the test compound during the corresponding urine-sampling period ($\tau$).

**PK-PD Model for the Effect of an SGLT Inhibitor on Renal Glucose Transport.**

The previously developed PK-PD model (Yamaguchi et al., 2011) was used in this study, and the details are shown below. To express the renal glucose reabsorption and the inhibitory effect of an SGLT inhibitor, the following concepts were assumed in the model: (1) the filtered plasma glucose is reabsorbed by SGLT2, and then the remainder is reabsorbed by SGLT1; (2) the flow rate in the proximal tubule is the same as the glomerular filtration rate (GFR); (3) the glucose concentration in the proximal tubule is altered only by the reabsorption mediated by SGLTs; (4) the glucose reabsorption process mediated by SGLTs is expressed by a nonlinear parallel tube model; (5) the inhibitor concentration in the proximal tubule is the same as that in plasma and shows a constant value; and (6) an inhibitor competitively inhibits the glucose transport mediated by SGLTs. A schematic representation of the PK-PD model is shown in Fig. 2. The glucose transport in the proximal tubule at time $t$ is expressed by the following equations:

$$C_{out2}(t) - C_{in2}(t) + K_{m,SGLT2} \cdot \left[1 + \frac{C(t)}{K_{SGLT2}}\right] \cdot \frac{\ln C_{out2}(t) - \ln C_{in2}(t)}{V_{max,SGLT2} \cdot GFR}$$

$$C_{out1}(t) - C_{in1}(t) + K_{m,SGLT1} \cdot \left[1 + \frac{C(t)}{K_{SGLT1}}\right] \cdot \frac{\ln C_{out1}(t) - \ln C_{in1}(t)}{V_{max,SGLT1} \cdot GFR}$$

$$C_{in2}(t) = C_{P,Glc}(t), \quad C_{out2}(t) = C_{in1}(t)$$

where Eqs. 4 and 5 express the glucose transport at the compartments of SGLT2 and SGLT1, respectively. $C_{in2}(t)$ is the glucose concentration just after filtration at time $t$, which is equal to the glucose concentration in plasma at time $t$ ($C_{P,Glc}(t)$). $C_{out2}(t)$ is the glucose concentration at the end of the SGLT2 compartment and is equal to $C_{in1}(t)$, which is the concentration at the entrance of the SGLT1 compartment at time $t$, $C_{out1}(t)$ is the concentration at the end of the SGLT1 compartment at time $t$. $C(t)$ is the plasma concentration of an inhibitor at time $t$ after administration. $K_{i,SGLT1}$ and $K_{i,SGLT2}$ are the inhibition constants of an inhibitor for SGLT1 and SGLT2, respectively. The plasma concentration-time profile of an inhibitor, $C(t)$, is assumed to be expressed on the basis of a 2-compartment model. Then, the following equation was obtained:

$$C(t) = \frac{Dose}{V_1} \cdot \left(\frac{(k_{21}-\alpha)}{(\beta-\alpha)} \cdot \exp(-\alpha \cdot t) + \frac{(k_{21}-\beta)}{(\alpha-\beta)} \cdot \exp(-\beta \cdot t)\right)$$

$$\alpha = \frac{(k_{10}+k_{12}+k_{21}) + \sqrt{(k_{10}+k_{12}+k_{21})^2 - 4 \cdot k_{21} \cdot k_{10}}}{2}$$

$$\beta = \frac{(k_{10}+k_{12}+k_{21}) - \sqrt{(k_{10}+k_{12}+k_{21})^2 - 4 \cdot k_{21} \cdot k_{10}}}{2}$$

where $Dose$ is the administration dose; $V_1$ is the distribution volume of the central compartment; $k_{12}$ and $k_{21}$ are the rate constants from central to peripheral and from peripheral to central compartment, respectively; $k_{10}$ is the elimination rate constant from the central compartment; and $\alpha$ and $\beta$ are the elimination rate constants at $\alpha$- and $\beta$-phase, respectively.

The renal glucose clearance at time $t$ ($|CL_{R,Glc}(t)|$) was estimated by the following equation:

$$CL_{R,Glc}(t) = GFR \cdot \frac{C_{out1}(t)}{C_{P,Glc}(t)}$$

The cumulative glucose amount excreted in urine at time $t$ ($|Q_{U,Glc}(t)|$) was estimated by the following equation:

$$Q_{U,Glc}(t) = \int_0^t CL_{R,Glc}(t) \cdot C_{P,Glc}(t)dt$$

**Estimation of Pharmacokinetic Parameters.** The plasma concentration-time profile of each test compound after intravenous administration to rat was analyzed on the basis of the 2-compartment model using WinNonlin, version 5.0, software (Pharsight Co., Mountain View, CA).

**PK-PD Analysis.** The time course of cumulative glucose excreted after intravenous administration of an SGLT inhibitor was analyzed on the basis of the PK-PD model with use of the following procedure. The $C(t)$ was simulated on the basis of the 2-compartment model using the PK parameters obtained from the present PK analysis. The $C_{P,Glc}(t)$ was calculated by linear interpolation between observed values. Next, the fixed values ($K_m, SGLT1$, $K_m, SGLT2$, $V_{max, SGLT1}$, $V_{max, SGLT2}$, and GFR) obtained from the previous study (Yamaguchi et al., 2011) and simulated values ($C(t)$ and $C_{P,Glc}(t)$) were substituted into Eqs. 4–6, 10 and 11. Then, the values for $Q_{U,Glc}(t)$ observed in the PK-PD study were fitted to Eq. 11 to estimate the parameters, rat $K_i$, SGLT1, and rat $K_i$, SGLT2. Curve-fitting procedures were performed using a nonlinear least square regression program, MULTI (http://www.pharm.kyoto-u.ac.jp/byoyaku/English/index.html) (Yamaoka et al., 1981), with weighting 1/5.

To compare in vivo $K_i$ with in vitro IC50, the in vitro IC50 values were transformed to $K_i$ values with use of the Cheng-Prusoff relationship (Cheng and Prusoff, 1973) as follows:

$$K_{i,SGLT1} = \frac{IC_{50,SGLT1}}{1 + \frac{S}{K_m,SGLT1}}$$

$$K_{i,SGLT2} = \frac{IC_{50,SGLT2}}{1 + \frac{S}{K_m,SGLT2}}$$

where $S$ means AMG concentration (1 mM) used in the in vitro SGLT inhibition study. Selectivity toward SGLT2 versus SGLT1 was calculated by dividing $K_i$, SGLT1 by $K_i$, SGLT2.

**Sensitivity Analysis.** To understand the relationship between selectivity toward SGLT2 and the effect on UGE, a simulation study was performed using the present PK-PD model. In the simulation, the
PD parameters relating to renal glucose transport were used (Table 2), and it was assumed that $K_i$,SGLT2 of an SGLT inhibitor was 1 nM and that the selectivity toward SGLT2 was in the range of 1–1000. In addition, no inhibition of SGLT1 (infinite selectivity toward SGLT2) was also assumed. CLR,Glc values were calculated under a static SGLT inhibitor concentration and at plasma glucose levels of 1, 2, or 3 mg/ml.

**Results**

**In Vitro IC$_{50}$ of SGLT Inhibitors.** The IC$_{50}$ values of test compounds determined from the AMG uptake assays are shown in Table 3. The IC$_{50}$ values for rSGLT2 of test compounds were in the range of 15–48 nM, and those for mSGLT1 were in the range of 970–27,000 nM. IC$_{50}$ values for mSGLT2 were in the range of 5.0–17 nM, and those for mSGLT1 were in the range of 96–6900 nM. Test compounds showed a large variety in IC$_{50}$ for mSGLT1 and rSGLT1, compared with mSGLT2 and rSGLT2. Of the test compounds, tofogliflozin exhibited the lowest IC$_{50}$ for both mSGLT2 (IC$_{50}$, 5.0 nM) and rSGLT2 (IC$_{50}$, 15 nM). Tofogliflozin, BMS, and sergliflozin-A showed relatively high selectivity toward SGLT2 (rat: more than 170; mouse: more than 150), and T-1095A and phlorizin showed less selectivity (rat: less than 20; mouse: less than 19). The rank orders of IC$_{50}$ values for both SGLT1 and SGLT2 and of selectivity toward SGLT2 in rat were the same as those in mouse.

**CLR,Glc After Administration of SGLT Inhibitors.** The relationship between CLR,Glc and $C_{AV,Drug}$ after administration of test compounds is plotted in Fig. 3. Tofogliflozin, BMS, and sergliflozin-A were administered intravenously and orally, and the plots obtained from both administration routes are overlapped for each compound (Fig. 3, A–C). The regression curve was estimated for each test compound with use of the plots of CLR,Glc values that were in the range of 50–400 ml/h/kg and using the following equation:

$$CL_{R,Glc} = \text{slope} \cdot \ln(C_{AV,Drug}) + a$$

The slopes of the regression curves for less selective SGLT2 inhibitors (T-1095A and phlorizin) were steeper than those for highly selective ones (tofogliflozin, BMS, and sergliflozin-A).

**PK-PD Analysis of SGLT Inhibitors for Renal Glucose Transport.** The effect of an SGLT inhibitor on UGE after intravenous administration was analyzed on the basis of the PK-PD model. The plasma concentration-time profile of each compound was suitably expressed using the 2-compartment model (Fig. 4), and the estimated parameters were summarized in Table 4. With use of the fixed PD parameters relating to glucose transport (Table 2) (Yamaguchi et al., 2011) and simulated plasma concentrations of compound and glucose, the curve-fitting of cumulative UGE for each compound was performed on the basis of the PK-PD model. The cumulative UGE of each test compound was suitably described by the present model (Fig. 4); then, $K_i$, SGLT1 and $K_i$, SGLT2 were estimated (Table 3).

**In Vitro-In Vivo Relationship of $K_i$ of SGLT Inhibitors.** The relationship between in vitro and in vivo $K_i$ values of SGLT inhibitors was analyzed using rat data. In vitro IC$_{50}$ values for SGLT1 and SGLT2 were transformed to $K_i$ values with use of the Cheng-Prusoff relationship (Cheng and Prusoff, 1973), and in vivo $K_i$ values were corrected by $f_w$.

The relationship between in vitro $K_i$ estimated using Cheng-Prusoff relationship and in vivo $K_i \times f_w$ is shown in Fig. 5A. In in vivo $K_i$, SGLT1 × $f_w$ values were in the range of 0.3–3.4-fold the in vitro data. The in vivo $K_i$, SGLT1 × $f_w$ values were in the range of 0.3–2.5-fold the in vitro data for three of four compounds and within 8-fold for one compound. In addition, the in vivo $K_i$, SGLT2 × $f_w$ values of test compounds were comparable to the in vitro data previously reported: in vitro $K_i$, SGLT2 values of tofogliflozin, sergliflozin, and phlorizin were 14.9 nM (Suzuki et al., 2012), 17.1 nM (Fujimori et al., 2009), and 39.4 nM (Suzuki et al., 2012), respectively. Moreover, there was a good correlation between in vitro and in vivo selectivity toward SGLT2 ($r = 0.985; P < 0.05$) (Fig. 5B).

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**TABLE 2**

Pharmacodynamic and biochemical parameters relating to rat renal glucose transport

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>GFR (ml/h/kg)</td>
<td>558</td>
<td>$K_{max}$,SGLT1 (mg/h/kg)</td>
<td>256</td>
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<tr>
<td>$V_{max}$,SGLT1</td>
<td>1392</td>
<td>$K_{max}$,SGLT2 (mg/ml)</td>
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<td>$K_{max}$,SGLT2</td>
<td>0.0862</td>
<td>$V_{max}$,SGLT2 (mg/ml)</td>
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<tr>
<td>$K_{max}$,SGLT1</td>
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**TABLE 3**

In vitro IC$_{50}$ and in vivo $K_i$ values for glucose transport mediated by SGLT1 and SGLT2, and plasma protein binding of test compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mouse</th>
<th>Rat</th>
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<tr>
<td></td>
<td>IC$_{50}$ (nM)</td>
<td>Selectivity toward SGLT2</td>
</tr>
<tr>
<td>Tofogliflozin</td>
<td>1,800</td>
<td>5.0</td>
</tr>
<tr>
<td>BMS</td>
<td>6,900</td>
<td>13 (49%)</td>
</tr>
<tr>
<td>Sergliflozin-A</td>
<td>990</td>
<td>6.8 (7%)</td>
</tr>
<tr>
<td>T-1095A</td>
<td>96</td>
<td>6.3 (8%)</td>
</tr>
<tr>
<td>Phlorizin</td>
<td>310</td>
<td>17</td>
</tr>
</tbody>
</table>

**Note:**
- NC, not calculated; NE, not examined.
- *Cited from Suzuki et al. (2012).
- $f_w = 2$
- $C_{AV,Drug}$

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Sensitivity Analysis. To understand how the selectivity toward SGLT2 of an SGLT inhibitor affects its inhibition of renal glucose reabsorption, a sensitivity analysis was performed: the fractional excretion of filtered glucose (FE) was simulated assuming 1 nM of $K_i$, SGLT2 value, different selectivity toward SGLT2, and several plasma glucose levels. In the case of euglycemic condition (1 mg/ml), the threshold inhibitor concentration that causes more than 0.1% of FE was 3.0 nM when selectivity was 1 and 6.5–8.0 nM when selectivity was in the range of 10 to infinity (Fig. 6A). The threshold concentration increased as selectivity toward SGLT2 increased. In the case of hyperglycemic condition (2 mg/ml), the threshold concentrations for different levels of selectivity toward SGLT2 were comparable, with values in the range of 1.5–2.0 nM (Fig. 6B).

Under euglycemic conditions, FE values showed a broad range between the different levels of selectivity toward SGLT2, with high selectivity causing a low FE value; FE value at a drug concentration of 1000 nM and at plasma glucose of 2 mg/ml was 96.2% when selectivity was 1 and was 76.0% when selectivity was infinity (Fig. 6B). FE value at a drug concentration of 1000 nM at plasma glucose of 1 mg/ml was 96.0% when selectivity was 1 and was 53.8% when selectivity was infinity (Fig. 6A). The difference in FE decreased as plasma glucose concentration increased.

Discussion

In the present study, a PK-PD analysis was performed on the basis of a nonlinear parallel tube model, in which different properties in glucose transport between SGLT1 and SGLT2 were considered, to estimate in vivo $K_i$ values and selectivity toward SGLT2 of SGLT inhibitors. The present PK-PD model was applied to SGLT inhibitors with different in vitro IC$_{50}$ values and selectivity toward SGLT2 and could suitably express their time courses of UGE after administration (Fig. 4). We previously reported that the PK-PD model could express the plasma glucose concentration–dependent renal glucose excretion and the inhibitory effect of a less selective inhibitor, phlorizin, on renal glucose reabsorption in normal rat (Yamaguchi et al., 2011). In the present study, it was found that this model could be used to analyze the effect of other inhibitors, including highly selective SGLT2 inhibitors, such as tofogliflozin. The in vivo $K_i$ × $f_a$ values for SGLT1 and SGLT2 estimated using the present PK-PD model were comparable to the in vitro values (Fig. 5A). Moreover, the in vivo selectivity toward SGLT2 showed a good correlation with that in vitro (Fig. 5B). These results suggest that in vitro inhibition potency is reflected in vivo. Although plasma and proximal tubular drug concentrations were assumed to be equal in the analysis, the assumption would not reflect the actual in vivo situation because of renal secretion or reabsorption of a drug; for example, when renal reabsorption of a drug occurs, the estimated inhibition constant based on plasma drug concentration would be higher than that based on the actual tubular concentration. A more accurate in vitro–in vivo relationship for inhibition potency might be obtained by considering tubular drug concentration.

When designing a potent SGLT2 inhibitor with specificity for a target organ, both inhibition potency to SGLT2 and selectivity toward SGLT2 are important indexes at the preclinical drug discovery stage. However, little is known of how the selectivity toward SGLT2 of a compound affects its inhibition potency for renal glucose reabsorption. In the
The present study, $CL_{R,Glc}$ was used to express the inhibitory effect of SGLT inhibitors on renal glucose reabsorption to evaluate the in vivo inhibition potency. As shown in Fig. 3, each inhibitor enhanced $CL_{R,Glc}$ as its plasma concentration increased, suggesting the plasma concentration–dependency of the inhibitory effect of SGLT inhibitors. The slope of the regression lines for the relationship between $CL_{R,Glc}$ and plasma inhibitor concentration obtained from highly selective SGLT2 inhibitors (tofogliflozin, BMS, and sergliflozin-A) was gradual, compared with that from less selective ones (T-1095A and phlorizin). Moreover, the maximum enhancing efficacy for $CL_{R,Glc}$ of highly selective inhibitors was lower than that of less selective ones. These facts suggest that highly selective SGLT2 inhibitors have a milder inhibitory effect on renal glucose reabsorption than less selective ones in euglycemic rats. Some investigators reported the same trend: a less selective SGLT inhibitor, phlorizin, showed complete inhibition of renal glucose reabsorption in human (Chasis et al., 1933) and dog (Silverman et al., 1970), but the highly selective SGLT2 inhibitors, dapagliflozin (Komoroski et al., 2009) and sergliflozin (Hussey et al., 2010), exhibited less than 50% inhibition in human.

As described above, it seems to be obvious that selectivity toward SGLT2 could affect the inhibition potency of a compound for renal glucose reabsorption. For a further understanding of the relationship between selectivity toward SGLT2 and inhibition potency for renal glucose reabsorption,

<table>
<thead>
<tr>
<th>Compound</th>
<th>$V_1$ (ml/kg)</th>
<th>$k_{10}$ (/h)</th>
<th>$k_{12}$ (/h)</th>
<th>$k_{21}$ (/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tofogliflozin</td>
<td>498 (23%)</td>
<td>2.40 (21%)</td>
<td>3.62 (34%)</td>
<td>1.08 (24%)</td>
</tr>
<tr>
<td>BMS</td>
<td>560 (19%)</td>
<td>1.30 (18%)</td>
<td>3.33 (38%)</td>
<td>1.39 (28%)</td>
</tr>
<tr>
<td>Sergliflozin-A</td>
<td>1820 (10%)</td>
<td>1.88 (10%)</td>
<td>1.70 (32%)</td>
<td>1.50 (29%)</td>
</tr>
<tr>
<td>T-1095A</td>
<td>1800 (15%)</td>
<td>1.30 (18%)</td>
<td>2.30 (33%)</td>
<td>0.965 (35%)</td>
</tr>
</tbody>
</table>

**Fig. 4.** Time courses of plasma concentrations for glucose and compound [tobogliflozin (A), BMS (B), sergliflozin-A (C), and T-1095A (D)] and of cumulative glucose excreted into urine after intravenous administration of each test compound. Open circle and error bar show the mean value of observed data ($n = 3$ or 4) and the corresponding S.D., respectively. Lines for PC and PG were simulated using PK parameters based on the 2-compartment model and linear interpolation, respectively. Line for GE was obtained by curve-fitting. GE, cumulative glucose excreted into urine; PC, plasma concentration of compound; PG, plasma concentration of glucose.

**TABLE 4**
Pharmacokinetic parameters of test compounds after intravenous administration.
PK parameters were estimated based on a 2-compartment model using WinNonlin Ver. 5.0 software (Pharsight Co., Mountain View, CA). The values in parentheses are CV of the estimate.
a simulation study based on the PK-PD model was performed, assuming several levels of selectivity toward SGLT2 under euglycemic and hyperglycemic conditions. The simulation results indicated that the inhibition potency of a highly selective SGLT2 inhibitor for renal glucose reabsorption was milder than that of a less selective one under euglycemic conditions (Fig. 6A). CL\textsubscript{R, Glc} observed in the present study (Fig. 3) and previous reports for UGE in the presence of SGLT2 selective and less selective inhibitors (Chasis et al., 1933; Komoroski et al., 2009; Nagata et al., 2012) support the simulation results under euglycemic conditions. On the other hand, the simulation results indicated that the inhibition potency of a highly selective SGLT2 inhibitor increased as plasma glucose level increased and that the difference in FE between inhibitors with different selectivity toward SGLT2 was slight under hyperglycemic conditions (Fig. 6, B and C). Nagata et al. (2012) reported that, in rats, a highly selective SGLT2 inhibitor, tofogliflozin, and a less selective one, phlorizin, showed 60 and 70% FE, respectively, at the unbound plasma concentrations (tofogliflozin: 196 nM; phlorizin: 1123 nM) under hyperglycemic conditions (300 mg/dl) but showed 20 and 60% FE, respectively, at the same concentrations under euglycemic conditions (100 mg/dl). Moreover, it was reported that the maximum renal glucose clearance induced by a highly selective SGLT2 inhibitor was comparable with that by a less selective inhibitor in db/db mice (Yamaguchi et al., 2012). These facts suggest the validity of the simulation under hyperglycemic conditions.

It was reported that a highly selective SGLT2 inhibitor, dapagliflozin, showed a low risk of hypoglycemia in clinical trials, because the overall incidence of major hypoglycemic events did not exceed 1% and was not significantly increased by dapagliflozin treatment; however, the risk of mild hypoglycemic events was more frequent with dapagliflozin than with the placebo (Musso et al., 2012). Most SGLT inhibitors in clinical development have high selectivity toward SGLT2; thus, the risk of hypoglycemia for a less selective SGLT inhibitor is not clear but may be higher than for a highly selective one, because a less selective one can inhibit both SGLT2 in the kidney and SGLT1 in both the kidney and the small intestine. Even if we focus on the action of an SGLT inhibitor only in the kidney, as shown in the simulation study, highly selective SGLT2 inhibition exhibiting mild FE under
euglycemic conditions (Fig. 6A) and great FE equal to a less selective one under hyperglycemic conditions (Fig. 6, B and C) would be a preferable characteristic for treating type II diabetes without incurring hypoglycemia, because there would be no necessity to reduce blood glucose level under euglycemic conditions for the treatment.

Although SGLT1 has been considered to make a minor contribution to renal glucose reabsorption (Abdul-Ghani et al., 2011), the complementary role of SGLT1 to SGLT2 has been recently stressed by some researchers (Hummel et al., 2011; Vallon et al., 2011) because of the following facts: (1) a highly selective SGLT2 inhibitor, dapagliflozin, caused only less than 50% inhibition of renal glucose reabsorption at the highest dose in healthy subjects (Komoroski et al., 2009), and (2) SGLT2 knockout mice excreted approximately 60% of filtered glucose into urine and reabsorbed 40% (Vallon et al., 2011). Therefore, the contribution of SGLT1 to renal glucose reabsorption is an interesting issue for understanding the efficacy of an SGLT inhibitor. The present PK-PD model could suitably express the phenomenon described above by assuming infinite selectivity toward SGLT2 of an SGLT inhibitor (i.e., no inhibition potency to SGLT1) under euglycemic conditions, as follows: the complete inhibition of SGLT2 caused 55% of FE, and the rest was reabsorbed by SGLT1 (Fig. 6A). This means that SGLT1 has transport capacity to reabsorb 45% of the filtered glucose under euglycemic condition when SGLT2 does not contribute to glucose reabsorption. Moreover, the simulation assuming infinite selectivity toward SGLT2 showed that FE was higher under hyperglycemic than under euglycemic conditions (Fig. 6, B and C). According to the assumptions of the PK-PD model, the difference in FE between euglycemic and hyperglycemic conditions can be simply explained as follows: FE value under impaired SGLT2 and saturated SGLT1 conditions is estimated by

\[
1 - \frac{V_{\text{max}}(\text{SGLT1})}{GFR}{\text{CF}_{\text{Glc}}} \times 100
\]

Thus, FE shows a higher value under hyperglycemic conditions than under euglycemic conditions. That would be the reason why a highly selective SGLT2 inhibitor could exhibit potent inhibition for renal glucose reabsorption under hyperglycemic conditions without inhibiting SGLT1. For the first time, the present PK-PD model enabled us to express the incomplete inhibition of renal glucose reabsorption by an SGLT2-selective inhibitor (Komoroski et al., 2009) and to explain why SGLT2 knockout mice could reabsorb 40% of the filtered glucose (Vallon et al., 2011) by considering both SGLT1 and SGLT2 contribution to renal glucose reabsorption; no complicated assumptions were required, such as secretion and/or reabsorption of an inhibitor in the proximal tubule (Liu et al., 2012). As we previously reported, the present PK-PD model using rat PD parameters could successfully simulate the excessive urinary glucose excretion in familial patients with renal glycosuria with SGLT2 mutation and the mild excretion in patients with glucose-galactose malabsorption with SGLT1 mutation (Yamaguchi et al., 2011). The previous simulation can also explain the recent finding of a slight increase of urinary glucose level in SGLT1 knockout mice (Gorboulev et al., 2012). It is interesting that the prediction of mouse and human from rat data were successful at a certain level; however, considering the possible species differences in GFR (Davies and Morris, 1993) and glucose transport capacity mediated by SGLTs, optimizing the PD parameters for each species would be preferable for a more accurate prediction. A PK-PD model considering only the function of SGLT2 for renal glucose reabsorption was reported to adequately capture the UGE caused by an SGLT2 inhibitor (Maurer et al., 2011), and the model can simply describe the effect of an SGLT2 inhibitor. However, the lack of SGLT1 function is likely to limit its usefulness. The present PK-PD model would be suitable for a comprehensive understanding of renal glucose movement and the inhibitory effect of an SGLT2 inhibitor on renal glucose reabsorption, because the model includes the differences between SGLT1 and SGLT2, not only in affinity and transport capacity for glucose but also in inhibition potency of a compound.

In conclusion, the present PK-PD model enabled us to analyze UGE induced by SGLT inhibitors with a wide range of selectivity toward SGLT2, and the estimated in vivo \( K_i \times f_u \) values for both SGLT1 and SGLT2 were comparable to the in vitro data. Thus, the model would be useful for predicting UGE caused by an SGLT inhibitor from in vitro inhibition potency and selectivity toward SGLT2.

Acknowledgments

The authors thank Drs. Naoki Taka and Tsutomu Sato (employees of Chugai Pharmaceutical Co., Ltd.) for assistance with synthesizing the test compounds; Dr. Masanori Fukazawa (Chugai Pharmaceutical Co., Ltd.) for good comments in preparing the manuscript; and Editing Services at Chugai Pharmaceutical Co., Ltd., for editing advice when preparing this article in English.

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Wrote or contributed to the writing of manuscript: Yamaguchi, Kato, Suzuki, Ishigui, Ikeda.

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