Absorption, Elimination, and Metabolism of CS-1036, a Novel α-Amylase Inhibitor in Rats and Monkeys, and the Relationship between Gastrointestinal Distribution and Suppression of Glucose Absorption

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ABSTRACT

The absorption, metabolism, and excretion of (2R,3R,4R)-4-hydroxy-2-(hydroxymethyl)pyrrolidin-3-yl 4-O-[(6-deoxy-β-D-glucopyranosyl)α-o-glucopyranoside (CS-1036), a novel and potent pancreatic and salivary α-amylase inhibitor, were evaluated in F344/DuCrjCrj rats and cynomolgus monkeys. The total body clearance and volume of distribution of CS-1036 were low (2.67-3.44 ml/min/kg and 0.218-0.237 l/kg for rats and 2.25-2.84 ml/min/kg and 0.217-0.271 l/kg for monkeys). After intravenous administration of [14C]CS-1036 to rats and monkeys, radioactivity was mainly excreted into urine (77.2% for rats and 81.1% for monkeys). After oral administration, most of the radioactivity was recovered from feces (80.28% for rats and 88.13% for monkeys) with a low oral bioavailability (1.73–2.44% for rats and 0.983–1.20% for monkeys). In rats, intestinal secretion is suggested to be involved in the fecal excretion as a minor component because fecal excretion after intravenous administration was observed (15.66%) and biliary excretion was almost negligible. Although intestinal flora was involved in CS-1036 metabolism, CS-1036 was the main component in feces (70.3% for rats and 48.7% for monkeys) and in the intestinal contents (33–68% for rats up to 2 hours after the dose) after oral administration. In Zucker diabetic fatty rats, CS-1036 showed a suppressive effect on plasma glucose elevation after starch loading with a 50% effective dose at 0.015 mg/kg. In summary, CS-1036 showed optimal pharmacokinetic profiles: low oral absorption and favorable stability in gastrointestinal lumen, resulting in suppression of postprandial hyperglycemia by α-amylase inhibition.

Introduction

CS-1036, (2R,3R,4R)-4-hydroxy-2-(hydroxymethyl)pyrrolidin-3-yl 4-O-[(6-deoxy-β-D-glucopyranosyl)α-o-glucopyranoside (Fig. 1), is a novel and potent inhibitor of pancreatic and salivary α-amylase in rats and humans (Honda et al., 2004). CS-1036 is expected to inhibit starch digestion in diets via α-amylase inhibition by oral administration, which then leads to the suppression of postprandial glucose absorption. As commercially available oral antidiabetic agents with similar mechanisms, the α-glucosidase inhibitors (α-GIs) acarbose, voglibose, and miglitol also suppress postprandial hyperglycemia by the inhibition of α-glucosidase expressed on intestinal brush border membranes (Martin and Montgomery, 1996; Hara and Hotta, 1997; Scott and Spencer, 2000). For the pharmacokinetic (PK) properties of α-GIs, orally administered miglitol is absorbed with a high oral bioavailability (Foral > 60%) and excreted into urine in unchanged form (Ahr et al., 1997). On the other hand, acarbose exhibits a low absorption and is metabolized by digestive enzymes and intestinal flora; urinary excretion of the unchanged form is below 3.4% of the dose (Ahr et al., 1989). The pharmacologic targets of CS-1036 are salivary and pancreatic α-amylase, which are secreted into saliva and pancreatic juices, respectively. Because the administration route of CS-1036 is oral, CS-1036 is expected to inhibit α-amylase in the gastrointestinal tract. From this perspective, low absorption and favorable stability are considered the optimal profile for CS-1036, which differs from the PK profile of the α-GIs.

In this study, the absorption, metabolism, gastrointestinal distribution, and excretion of CS-1036 were investigated in F344/DuCrjCrj

**Abbreviations:** α-GI, α-glucosidase inhibitor; AUC, area under the plasma concentration versus time curve; AUCoral, area under the plasma concentration versus time curve up to the last quantifiable time; AUCint, area under the plasma concentration versus time curve up to infinity; ΔAUCoral, area under the curve of change of plasma glucose level normalized by the plasma glucose level at 0 hours as a baseline; ΔAUCoral_min, area under the curve of change of plasma glucose level normalized by the plasma glucose level at 0 h as a baseline in the nonstarch control group; BDC, bile duct-cannulated; CI, confidence interval; CL, total body clearance; Cmax, maximum plasma concentration; CS-1036, (2R,3R,4R)-4-hydroxy-2-(hydroxymethyl)pyrrolidin-3-yl 4-O-[(6-deoxy-β-D-glucopyranosyl)α-o-glucopyranoside; DAB, 1,4-dideoxy-1,4-imino-D-arabinitol; FA, oral bioavailability; F344, F344/DuCrjCrj; γ, sigmoidicity factor; GP, glycogen phosphorylase; HPLC, high-performance liquid chromatography; IC50, 50% inhibitory concentration; t1/2, terminal elimination rate constant; LC-MS/MS, liquid chromatography–mass spectrometry; LC-MS/MS, liquid chromatography–tandem mass spectrometry; PK, pharmacokinetic; PYF, peptone yeast extract Fildes solution; RB, blood/plasma ratio; tmax, time to reach maximum plasma concentration; Vdss, volume of distribution at steady state; ZDF, Zucker diabetic fatty.
Pharmacokinetics of CS-1036 in Rats and Monkeys

(F344) rats and cynomolgus monkeys. To confirm the assumption that intestinal concentrations of CS-1036 affect its inhibitory effects on starch digestion, the suppression of plasma glucose elevation was investigated in Zucker diabetic fatty (ZDF, ZDF/Crl-Lepfa) rats after starch loading.

Materials and Methods

Materials

CS-1036 and its metabolites M1, M2, and M3 (Supplemental Figs. 1, 2, and 3) were synthesized at Daiichi Sankyo Co., Ltd. (Tokyo, Japan) according to the published procedures (Honda et al., 2004). The internal standards, [3H]–CS-1036 and [2H]–M1 (Fig. 1), for the quantification of CS-1036 and M1 were also synthesized at Daiichi Sankyo Co., Ltd. [14C]CS-1036 (41.9, 52.2, 42.2, and 63.5 µCi/mg: four different lots) was synthesized at GE Healthcare UK Ltd. (Buckinghamshire, UK) and Sekisui Medical Co., Ltd. (Tokyo, Japan). The radiochemical purities of [14C]CS-1036 were guaranteed to be more than 98% at synthesis and more than 95% in the experiments by high-performance liquid chromatography (HPLC) with radioactive flow detection. All other reagents and solvents used were commercially available and were of extra pure, guaranteed, HPLC or liquid chromatography-mass spectrometry (LC-MS) grade.

Animals

All animal studies were conducted with approval in accordance with the guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo. Male F344 rats at 7 weeks of age and male ZDF rats at 6 weeks of age were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). F344 rats were used after acclimatization of at least 5 days. The ZDF rats were acclimatized until they were 10 weeks of age. Male cynomolgus monkeys purchased from Japan Laboratory Animals, Inc. (Tokyo, Japan) or Guangxi Grandforest Scientific Primate Company, Ltd. (Guangxi, China) were used at 2–5 years of age after quarantine and acclimatization for more than 6 weeks. Diets were freely accessed by rats and were supplied once daily to monkeys. Water was provided ad libitum throughout the experiments. All the animals were fasted overnight before administration and through to 4–8 hours after dosing.

Pharmacokinetics of CS-1036 in Rats and Monkeys

CS-1036 (0.3, 1.0, 3.0, and 10.0 mg/kg) was administered intravenously or orally to fasted F344 rats (146–185 g, n = 4 each) and monkeys (3.48–4.29 kg, n = 4 each). The monkey study was conducted with 2-week washout periods between doses. Blood was collected at designated time points up to 48 hours after the dose. Plasma was obtained by centrifugation at 4°C and was stored at −70°C until analysis.

Mass Balance Study in Rats

[14C]CS-1036 (1 mg/kg, 41.9 µCi/kg) was administered intravenously via the tail vein (152–156 g, n = 4) or orally (154–159 g, n = 4) to fasted F344 rats. After administration, the rats were housed individually in metabolic cages, and urine and feces were collected at designated intervals up to 168 hours after the dose. Carbon dioxide in the expired air was trapped by the mixture of 2-aminoethanol/2-methoxyethanol = 1/1 (v/v) at designated intervals up to 48 hours after the dose in rats.

Biliary Excretion of Radioactivity in Bile Duct-Cannulated (BDC) Rats

Fasted F344 rats were subjected to cannulation with a flexible polyethylene tube (SP-31; Natsume Seisakusho Co., Ltd., Tokyo, Japan) into the common bile duct and fixed by placing a ligature around the tube to prevent dislocation under diethyl ether anesthesia. After recovery from the anesthesia, the dosing solution at a dose of 1 mg/kg (41.9 µCi/kg) of [14C]CS-1036 was administered intravenously via the tail vein (161–165 g, n = 4) or by oral gavage (157–162 g, n = 4). The rats were individually accommodated in Bollman cages, and bile was collected in tubes at 0–6, 6–24, 24–30, and 30–48 hours in a water bath set at 4°C.

Mass Balance Study in Monkeys

[14C]CS-1036 (3 mg/kg, 191 and 156.5 µCi/kg for intravenous and oral administration, respectively) was administered intravenously via the saphenous vein (3.85–4.75 kg, n = 4) or orally by a stomach catheter (3.77–4.18 kg, n = 4) to fasted monkeys. After administration, urine, feces, and cage-washing solution with water were collected at designated intervals up to 336 hours after the dose.

Sample Preparation for Qualitative In Vivo Metabolite Analysis in Urine and Feces

[14C]CS-1036 (3 mg/kg, 126 µCi/kg) was administered orally by a stomach catheter to fasted F344 rats (148–176 g, n = 3) and monkeys (2.44–3.14 kg, n = 3). Urine and feces were collected up to 24 and 48 hours after the dose from rats and monkeys, respectively. Urine was applied to solid phase extraction using Oasis MCR cartridges (60 mg, 3 ml; Waters Corp., Milford, MA), which had been preconditioned with 3 ml of methanol and 3 ml of water. The cartridges were washed with 1 ml of water followed by the elution of radioactive components with 3 ml of 28% ammonia solution/methanol (5/95, v/v). Feces were homogenized with water (50 ml for rats and 4-fold volume of feces for monkeys), and extracted with an equal volume of methanol for rats and with 2-fold volume of methanol for monkeys. The fecal extracts were applied to Oasis HLB cartridges (60 mg, 3 ml; Waters Corp.), which had been preconditioned with 3 ml of methanol and 3 ml of water. The radioactive components were eluted with 0.5 ml of water followed by 3 ml of methanol. Pretreated urinary and fecal samples were lyophilized and reconstituted with 0.1–0.4 ml of water.

Sample Preparation for In Vitro Qualitative Metabolite Analysis

Hepatic, Renal, and Intestinal S9 Fractions. [14C]CS-1036 (final concentration: 22.7 µM, 0.42 µCi/ml) was incubated at 37°C for 1 hour in 2 mg protein/ml of hepatic, renal, and intestinal S9 fractions from rats, monkeys, and humans in 100 mM potassium phosphate buffer (pH 7.4) with an NADPH-generating system (2.5 mM NADP, 25 mM glucose-6-phosphate, 0.5 units/ml glucose-6-phosphate dehydrogenase, 10 mM magnesium chloride). After incubation, 0.2 ml of the reaction mixture was mixed with 0.2 ml of acetonitrile for the termination of the reaction. The supernatant obtained by centrifugation was subjected to metabolite analysis.

Anaerobic Culture Broth of Rat Cecal Contents and Feces from Monkeys and Humans. After the F344 rats were sacrificed, the cecum was removed, and the cecal contents were collected (n = 4). The feces were collected using a sterile swab from the monkeys (n = 4) and healthy male volunteers (n = 5, 29–54 years of age). The collected cecum and feces were maintained with oxygen-absorbing and carbon dioxide-generating agents in a hermetically sealed pouch at room temperature until use. The rat cecal contents and the monkey and human feces were suspended in 5 ml of peptone yeast extract Fildes solution (PYF) broth within 24 hours of collection. The suspensions of rat cecal contents and
Sample Preparation for Quantitative Analysis of Radioactivity in Urine and Feces

\[^{14}\text{C}]\text{CS-1036} (1 \text{ mg/kg, } 42.2 \mu\text{Ci/kg}) was administered orally to fasted F344 rats (164–168 g, \(n = 3\)). Urine and feces were collected up to 24 hours after the dose administration. In monkeys, samples for in vivo qualitative metabolite analysis were also used for quantitative metabolite analysis in urine and feces. Rat feces were homogenized with 9-fold volumes of water. The fecal homogenate was mixed with the same volume of methanol for rats and 2-fold volumes of methanol for monkeys. The supernatant after centrifugation was used for the analysis.

Sample Preparation for Quantitative Analysis of Radioactivity in Gastrointestinal Contents in Rats

\[^{14}\text{C}]\text{CS-1036} (1 \text{ mg/kg, } 42.2 \mu\text{Ci/kg}) was administered orally to fasted F344 rats (148–168 g, \(n = 3\)). The stomach, duodenum and jejunum, ileum, and cecum were observationally isolated, and the gastrointestinal contents were collected at 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 hours after the dose administration by washing with 5 ml of water 3 times (total: 15 ml). Each of the gastrointestinal content collections was homogenized, mixed with the same volume of acetonitrile, and the same volume of methanol for rats and 2-fold volumes of methanol for monkeys. The supernatant after centrifugation was used for the analysis.

Blood/Plasma Ratio of \[^{14}\text{C}]\text{CS-1036}

Aliquots of 5 \mu l of \[^{14}\text{C}]\text{CS-1036} solution (final concentrations: 113, 227, 1130, 2270, and 11300 nM) were mixed with 495 \mu l of rat, monkey, or human blood, and were incubated for 5 minutes at 37°C. The radioactivity of the blood was collected, and the residual extract was freeze-dried. The resulting residues from the contents of the stomach, duodenum and jejunum, ileum were reconstituted by 50% methanol (0.2 ml), respectively. Each resulting solution was centrifuged (4°C, 10,000g, 10 minutes) to obtain the supernatant as an HPLC sample.

TABLE 1
Pharmacokinetic parameters of CS-1036 after a single intravenous administration of CS-1036 to rats and monkeys

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>AUC_{int} (ng·h/ml)</th>
<th>AUC_{0→t} (ng·h/ml)</th>
<th>CL (ml/min/kg)</th>
<th>Vss (l/kg)</th>
<th>t_{1/2} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.3</td>
<td>1860 ± 290</td>
<td>1870 ± 290</td>
<td>2.73 ± 0.46</td>
<td>0.218 ± 0.038</td>
<td>14.6 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4920 ± 670</td>
<td>4930 ± 670</td>
<td>3.44 ± 0.51</td>
<td>0.237 ± 0.038</td>
<td>18.4 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18,700 ± 1300</td>
<td>18,800 ± 1300</td>
<td>2.67 ± 0.17</td>
<td>0.219 ± 0.020</td>
<td>23.6 ± 6.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>53,000 ± 5600</td>
<td>53,200 ± 5600</td>
<td>3.16 ± 0.37</td>
<td>0.222 ± 0.025</td>
<td>21.5 ± 1.6</td>
</tr>
<tr>
<td>Monkey</td>
<td>0.3</td>
<td>2000 ± 350</td>
<td>2010 ± 350</td>
<td>2.55 ± 0.47</td>
<td>0.233 ± 0.029</td>
<td>2.51 ± 1.06</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5870 ± 310</td>
<td>5890 ± 310</td>
<td>2.84 ± 0.15</td>
<td>0.271 ± 0.024</td>
<td>21.4 ± 10.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18,100 ± 800</td>
<td>18,200 ± 800</td>
<td>2.75 ± 0.12</td>
<td>0.262 ± 0.025</td>
<td>25.1 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>74,300 ± 7300</td>
<td>74,500 ± 7300</td>
<td>2.25 ± 0.22</td>
<td>0.217 ± 0.019</td>
<td>30.0 ± 4.6</td>
</tr>
</tbody>
</table>
Sample Analysis

Quantification of CS-1036 and M1. Plasma concentrations of CS-1036 and M1 were determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS). For the PK study, the plasma sample (50 μl), including study samples or control plasma (for standard and quality controls), was mixed with 50 μl of IS (100 ng/ml of 3H2-CS-1036 and 100 ng/ml of 3H2-M1 in 50% methanol), 50 μl of 50% methanol for study samples or standard or quality control samples in 50% methanol and 0.8 ml of water. The sample then was subjected to a solid-phase extraction (100 mg, VersaPlate SCX; Agilent Technologies, Santa Clara, CA). The extracted sample was evaporated by nitrogen stream, and the residue was dissolved in 180 μl of mobile phase A (as described later), and 20 μl of the sample was injected to an LC-MS/MS system consisting of an API4000 mass spectrometer (Applied Biosystems, Foster City, CA) coupled to an HPLC system (Agilent 1100 series HPLC, Agilent Technologies; or 2795 separations module; Waters Corp.). The analytes were separated on a Capcell-pak UG80 SCX (5 mm, 250 mm, 2.0 μl) column; they were then analyzed with a liquid scintillation analyzer. The resulting 14CO2 was absorbed in Carbo-Sorb E (6 ml; Waltham, MA; or Cleasol I; Nacalai Tesque); they were then analyzed with a liquid scintillation analyzer. The solvent trapped expired air (1 ml) was mixed with a scintillation cocktail (10 ml of Hionic-Fluor), and then analyzed with a liquid scintillation counter. For fecal metabolite profiling studies in rats, the fecal sample was mixed with water of approximately 9-fold volume of the sample weight and then homogenized. The fecal homogenate (0.5 ml) was placed on a combust pad and weighed; then it was combusted with a sample oxidizer. The resulting 14CO2 was absorbed in Carbo-Sorb E (6 ml; PerkinElmer), mixed with a scintillation cocktail (9 ml, Permafluor+E; PerkinElmer), and then analyzed with the liquid scintillation analyzer. The solvent trapped expired air (1 ml) was mixed with a scintillation cocktail (10 ml of Hionic-Fluor) and then analyzed with a liquid scintillation counter.

Qualitative and Quantitative Metabolite Profiling by HPLC with Radioactivity Detector and LC-MS. Metabolites in urine, feces, gastrointestinal contents, and in vitro samples were analyzed using an HPLC system (Alliance 2695 separations module equipped with a 2996 photodiode array detector; Waters Corp.) equipped with a mass spectrometer (Q-TOF Ultima mass spectrometer; Waters Corp.), or an LC-10Avp HPLC system (Shimadzu Corp., Kyoto, Japan) or an LC-2000 HPLC system (Hitachi High-Technologies Corp., Tokyo, Japan) equipped with a Radiomatic 625TR radioactivity detector (PerkinElmer), and a mass spectrometer (Accela-LTQ Orbitrap XL; Thermo Fisher Scientific, Waltham, MA). The analytical conditions for HPLC and radioactivity detector were as follows: analytical column, Atlantis HILIC Silica (5 μm, 4.6 × 250 mm; Waters Corp.); column oven temperature, 30°C; mobile phase A, 0.1% (v/v) trifluoroacetic acid in water; mobile phase B, 0.1% (v/v) trifluoroacetic acid in acetonitrile; flow rate, 1 ml/min; gradient of mobile phase B, 95% from zero to 5 minutes (constant), 95 to 75% for 5 to 25 minutes (linear), 75 to 50% for 25 to 30 minutes (linear), and 50 to 5% for 30 to 35 minutes (linear).

Radioactivity Analysis. Plasma (0.1 ml), urine (0.1–1 ml), and bile (25–100 μl) were mixed with tissue solubilizer (1 ml of NCS-II; GE Healthcare UK Ltd.; or 1 ml of tissue solubilizer for BIOMERIT; Nacalai Tesque, Japan) and scintillation cocktail (10 ml of Hionic-Fluor; PerkinElmer, Tokyo, Japan); or Cleasol I (Nacalai Tesque); aliquots (0.5 ml) were placed on a combust pad and weighed; they then were combusted with a sample oxidizer. The resulting 14CO2 was absorbed in Carbo-Sorb E (6 ml; PerkinElmer), and then analyzed with the liquid scintillation analyzer. The solvent trapped expired air (1 ml) was mixed with a scintillation cocktail (10 ml of Hionic-Fluor) and then analyzed with a liquid scintillation counter. For fecal metabolite profiling studies in rats, the fecal sample was mixed with water of approximately 9-fold volume of the sample weight and then homogenized. The fecal homogenate (0.5 ml) was placed on a combust pad and weighed; then it was combusted with a sample oxidizer. The resulting 14CO2 was absorbed in Carbo-Sorb E (6 ml; PerkinElmer), mixed with a scintillation cocktail (9 ml, Permafluor+E; PerkinElmer), and then analyzed with the liquid scintillation analyzer. The solvent trapped expired air (1 ml) was mixed with a scintillation cocktail (10 ml of Hionic-Fluor) and then analyzed with a liquid scintillation counter.

Pharmacokinetic parameters of CS-1036 after a single oral administration of CS-1036 to rats and monkeys

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>AUCmax</th>
<th>AUCinf</th>
<th>tmax</th>
<th>Cmax</th>
<th>t1/2</th>
<th>Foral (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.3</td>
<td>42.1 ± 13.1</td>
<td>45.6 ± 17.2</td>
<td>0.500 ± 0.354</td>
<td>16.2 ± 2.4</td>
<td>2.69 ± 2.55</td>
<td>2.44</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>110 ± 26</td>
<td>115 ± 30</td>
<td>0.417 ± 0.144</td>
<td>45.5 ± 5.5</td>
<td>1.81 ± 0.31</td>
<td>2.33</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>324 ± 25</td>
<td>325 ± 29</td>
<td>0.375 ± 0.144</td>
<td>113 ± 15</td>
<td>4.16 ± 1.94</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1050 ± 140</td>
<td>1080 ± 150</td>
<td>0.438 ± 0.375</td>
<td>285 ± 75</td>
<td>18.3 ± 6.7</td>
<td>2.03</td>
</tr>
<tr>
<td>Monkey</td>
<td>0.3</td>
<td>15.0 ± 4.2</td>
<td>—</td>
<td>1.75 ± 0.50</td>
<td>5.23 ± 1.24</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>52.0 ± 11.4</td>
<td>57.9 ± 8.2</td>
<td>1.75 ± 0.50</td>
<td>15.3 ± 4.2</td>
<td>1.29 ± 0.19</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>186 ± 67</td>
<td>191 ± 71</td>
<td>1.75 ± 0.50</td>
<td>44.8 ± 11.0</td>
<td>2.12 ± 0.47</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>869 ± 300</td>
<td>894 ± 305</td>
<td>1.75 ± 0.50</td>
<td>132 ± 77</td>
<td>12.6 ± 5.0</td>
<td>1.20</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. (n = 3 or 4), and a dash denotes not applicable.

Excretion of radioactivity after single intravenous and oral administrations of 14C-CS1036 to rats and monkeys

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose/Route</th>
<th>Collection Period</th>
<th>h</th>
<th>% of dose</th>
<th>Urine</th>
<th>Feces</th>
<th>Expired Air</th>
<th>Bile</th>
<th>Cage Washing</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>1 mg/kg, i.v.</td>
<td>0–48</td>
<td>70.49 ± 7.96</td>
<td>15.66 ± 8.04</td>
<td>0.82 ± 1.05</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>86.96 ± 5.92</td>
</tr>
<tr>
<td></td>
<td>0–168</td>
<td></td>
<td>77.21 ± 7.62</td>
<td>17.82 ± 8.83</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>95.85 ± 4.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mg/kg, oral</td>
<td>0–48</td>
<td>13.34 ± 3.53</td>
<td>77.59 ± 2.84</td>
<td>2.34 ± 0.74</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>93.26 ± 1.53</td>
</tr>
<tr>
<td></td>
<td>0–168</td>
<td></td>
<td>15.32 ± 3.09</td>
<td>80.28 ± 3.88</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>97.94 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>BDC rat</td>
<td>1 mg/kg, i.v.</td>
<td>0–48</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.37 ± 0.08</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>1 mg/kg, oral</td>
<td>0–48</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.59 ± 0.04</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.59 ± 0.04</td>
</tr>
<tr>
<td>Monkey</td>
<td>3 mg/kg, i.v.</td>
<td>0–336</td>
<td>80.13 ± 4.76</td>
<td>0.46 ± 0.16</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>13.70 ± 4.19</td>
<td>94.29 ± 1.08</td>
</tr>
<tr>
<td></td>
<td>0–336</td>
<td></td>
<td>81.13 ± 4.92</td>
<td>0.66 ± 0.18</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>14.50 ± 4.04</td>
<td>96.29 ± 1.24</td>
</tr>
<tr>
<td></td>
<td>3 mg/kg, oral</td>
<td>0–48</td>
<td>4.54 ± 2.23</td>
<td>78.50 ± 8.09</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.76 ± 0.37</td>
<td>83.80 ± 8.88</td>
</tr>
<tr>
<td></td>
<td>0–48</td>
<td></td>
<td>5.67 ± 2.33</td>
<td>88.13 ± 3.04</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.35 ± 0.79</td>
<td>95.15 ± 1.48</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. (n = 4), and a dash denotes not tested.
The plasma glucose level (time point in the corresponding animal. The area under the curve of change in calculated by subtracting the plasma glucose level at 0 hours from that at each time point for each group. The ...distribution at a steady state ($V_{ss}$) was calculated as the dose AUMC/AUC$_{0\text{-}ss}$, where AUMC is the area under the first moment of the plasma concentration-time curve. The $t_{1/2}$ for each dose was calculated by dividing the AUC$_{0\text{-}ss}$ for the oral dose by that for the intravenous dose at the same dose level.

**Evaluation of Plasma Glucose Levels**

The changes of plasma glucose levels after the dose administration were calculated by subtracting the plasma glucose level at 0 hours from that at each time point in the corresponding animal. The area under the curve of change in the plasma glucose level ($\Delta$AUC$_{pg}$), which was normalized by the plasma glucose level at 0 hours as a baseline, was calculated by the trapezoidal method. The ED$_{50}$ and its 95% confidence intervals (CI) were estimated according to the sigmoid $E_{max}$ model described here, where the $\Delta$AUC$_{pg}$ in the starch control group and the $\Delta$AUC$_{pg}$ in the nonstarch control group were substituted for the upper limit (\(\Delta\)AUC$_{pg\text{-max}}$) and lower limit (\(\Delta\)AUC$_{pg\text{-min}}$) of the model, respectively. The sigmoidicity factor ($\gamma$) and its 95% CI were also estimated by SAS System Release 8.2 (SAS Institute, Inc., Cary, NC):

\[
\Delta\text{AUC}_{pg} = \Delta\text{AUC}_{pg\text{-min}} + \frac{(\Delta\text{AUC}_{pg\text{-max}} - \Delta\text{AUC}_{pg\text{-min}}) \times [\text{dosage}]^\gamma}{[\text{ED}_{50}]^\gamma + [\text{dosage}]^\gamma}
\]

**Statistical Analysis**

For the plasma glucose levels and the changes of plasma glucose levels, the summary statistics (n, arithmetic mean, S.E., and 95% CI) were calculated at each time point for each group. The $\Delta$AUC$_{pg}$ in the groups treated with CS-1036 was compared with that of the starch control group by a Dunnett test. $P < 0.05$ was considered statistically significant.

**Results**

**Pharmacokinetics of CS-1036 in Rats and Monkeys.** The plasma concentration-time profiles of CS-1036 in rats and monkeys after intravenous and oral administration (10 mg/kg) are shown in Fig. 2. The PK parameters of CS-1036 in rats and monkeys after intravenous and oral administration are shown in Tables 1 and 2, respectively. For intravenous administration, CS-1036 was disposed in a biphasic manner in both species. The CL and $V_{ss}$ were similarly low for both species: 2.67–3.44 ml/min/kg and 0.218–0.237 l/kg for rats and 2.25–2.84 ml/min/kg and 0.217–0.271 l/kg for monkeys. The AUC exhibited a dose-proportional increase in rats and monkeys (0.3–10 mg/kg). For oral administration, AUC and $C_{max}$ also exhibited a dose-dependent increase in rats and monkeys (0.3–10 mg/kg) although the dose-normalized AUC and $C_{max}$ in rats after oral administration were slightly higher at 0.3 mg/kg and the dose-normalized AUC in monkeys after oral administration tended to increase with each dose. The $t_{max}$ was 0.375–0.5 hours for rats and 1.75 hours for monkeys.
The mean $F_{\text{oral}}$ was 1.73%–2.44% for rats and 0.983%–1.20% for monkeys.

**Excretion of Radioactivity.** The excretion of radioactivity after intravenous and oral administration of $[^{14}\text{C}]$CS-1036 to rats and monkeys is summarized in Table 3. After intravenous administration, most of the radioactivity was recovered from feces for both species. In monkeys, the fecal excretion was 88.13% of the dose up to 336 hours after the dose mainly as a result of unabsorbed radioactivity. In rats, the fecal excretion was 80.28% of the dose up to 168 hours after the dose, in which the component of intestinal secretion might be included in addition to the unabsorbed radioactivity because fecal excretion was observed (15.66% of the dose) after intravenous administration and biliary excretion in BDC rats was almost negligible (0.37% of the intravenous administration and 0.59% of the oral administration up to 48 hours after the dose). The urinary excretion of radioactivity was 15.32% and 5.67% of the dose for rats and monkeys, respectively. In rats, the radioactivity excreted into the expired air was higher after oral administration (2.34% of the dose) than intravenous administration (0.82% of the dose).

**In Vivo Metabolite Identification.** Representative radiochromatograms of the metabolites in rat and monkey urine and feces after oral administration of $[^{14}\text{C}]$CS-1036 are shown in Fig. 3. In rat and monkey urine, M1 was detected as a metabolite. The positive ion LC-MS spectrum of M1 showed a protonated molecule [M+H]$^+$ at m/z 134, and was consistent with its authentic standard. M1 was identified as 1,4-dideoxy-1,4-imino-D-arabinitol (DAB). In rat and monkey feces, M1 and M2 were detected as metabolites. The positive ion LC-MS spectrum of M2 showed a protonated molecule [M+H]$^+$ at m/z 176, and was consistent with its authentic standard. M2 was identified as N-acetylated M1.

**In Vitro Metabolism of CS-1036.** To identify whether the liver, kidney, and small intestine are responsible for CS-1036 biotransformation, an in vitro metabolism study using these S9 fractions was performed. Incubation of $[^{14}\text{C}]$CS-1036 with the hepatic, renal, or intestinal S9 fraction from rats, monkeys, or humans in the presence of NADPH did not produce any metabolites (Supplemental Fig. 4). Therefore, we supposed that in vivo biotransformation of CS-1036—namely, hydrolysis followed by N-acetylation—might be mediated by the intestinal flora in the intestine. To confirm this, CS-1036 was incubated in a culture broth derived from rat cecal contents and was identified as an N-acetylated M1.

**Quantitative Plasma Profiles of M1 in Rats and Monkeys.** After oral administration of $[^{14}\text{C}]$CS-1036 to rats and monkeys, we did not observe metabolites in the radiochromatogram of the plasma samples for rats and 81.13% of the dose up to 336 hours after the dose for monkeys. Species differences of fecal excretion of radioactivity were observed, and the radioactivity recovered from feces was higher in rats (17.82% of the dose) than monkeys (0.66% of the dose). After oral administration, most of the radioactivity was recovered from feces for both species. In monkeys, the fecal excretion was 88.13% of the dose up to 336 hours after the dose mainly as a result of unabsorbed radioactivity. In rats, the fecal excretion was 80.28% of the dose up to 168 hours after the dose, in which the component of intestinal secretion might be included in addition to the unabsorbed radioactivity because fecal excretion was observed (15.66% of the dose) after intravenous administration and biliary excretion in BDC rats was almost negligible (0.37% of the intravenous administration and 0.59% of the oral administration up to 48 hours after the dose). The urinary excretion of radioactivity was 15.32% and 5.67% of the dose for rats and monkeys, respectively. In rats, the radioactivity excreted into the expired air was higher after oral administration (2.34% of the dose) than intravenous administration (0.82% of the dose).

![Radioactivity Excretion](image)

**TABLE 4**

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>$t_{\text{max}}$ (h)</th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>$AUC_{\text{last}}$ (ng/ml*h)</th>
<th>$%$ of CS-1036</th>
<th>Ratio of Molar-Based $AUC_{\text{last}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>i.v.</td>
<td>3</td>
<td>0.354 ± 0.438</td>
<td>0.774 ± 0.231</td>
<td>0.387 ± 0.169</td>
<td>0.00672 ± 0.00257</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.125 ± 0.083</td>
<td>2.67 ± 0.57</td>
<td>2.82 ± 1.59</td>
<td>0.0172 ± 0.0086</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>oral</td>
<td>0.3</td>
<td>1.25 ± 0.50</td>
<td>1.71 ± 0.21</td>
<td>2.64 ± 1.32</td>
<td>22.1 ± 12.6</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>1.33 ± 0.58</td>
<td>10.6 ± 5.1</td>
<td>26.1 ± 18.6</td>
<td>73.4 ± 34.9</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0.688 ± 0.375</td>
<td>20.9 ± 3.5</td>
<td>49.2 ± 12.2</td>
<td>49.9 ± 9.6</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.938 ± 0.774</td>
<td>39.8 ± 6.5</td>
<td>103 ± 33</td>
<td>32.2 ± 8.4</td>
<td>9.6</td>
</tr>
<tr>
<td>Monkey</td>
<td>oral</td>
<td>1</td>
<td>3.50 ± 1.91</td>
<td>1.40 ± 0.52</td>
<td>9.66 ± 4.93</td>
<td>60.8 ± 26.6</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1.75 ± 0.50</td>
<td>4.86 ± 1.29</td>
<td>40.2 ± 11.0</td>
<td>83.5 ± 54.0</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>1.25 ± 0.50</td>
<td>20.7 ± 7.0</td>
<td>146 ± 22</td>
<td>62.5 ± 28.1</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Fig. 4. Representative radiochromatograms obtained by analysis of the culture broths of rat cecal contents, monkey feces, and human feces after incubation of $[^{14}\text{C}]$CS-1036 under anaerobic conditions for 24 hours.
owing to the detection limit (unpublished data), but M1 was observed in urinary samples. Thereafter, we determined M1 concentrations in plasma by LC-MS/MS using the reference standard of M1 and its internal standard. The PK parameters of M1 in rats and monkeys are shown in Table 4. After a single intravenous administration of CS-1036, M1 was detected at low levels in rats at doses of 3 and 10 mg/kg. M1 was not detected at the lower doses (0.3 and 1 mg/kg) in rats or at any dose levels (0.3–10 mg/kg) in monkeys. After a single oral administration of CS-1036, we observed the \( t_{\text{max}} \) of M1 at 0.688–1.33 hours after the dose in rats and at 1.25–3.5 hours after the dose in monkeys. The molar-based ratio of the AUC of M1 to that of CS-1036 ranged from 22.1 to 83.5% after oral administration of CS-1036 to rats and monkeys.

**Quantitative Metabolite Profiles of Urine and Feces.** The quantitative metabolite profiles in urine and feces up to 24 hours for rats and up to 48 hours for monkeys after oral administration of \([^{14}\text{C}]\text{CS-1036}\) are summarized in Table 5. In urine, CS-1036 (1.4% of the dose for rats and 2.9% of the dose for monkeys) and M1 (5.3% of the dose for rats and 2.2% of the dose for monkeys) were detected. In feces, a species difference in the metabolite formation was observed; M1 and M2 were detected not in rats but in monkeys (9.7 and 17.8% of the dose, respectively), although M1 and M2 were identified in rat feces during the in vivo metabolite identification.

**Quantitative Metabolite Profiles of Gastrointestinal Contents in Rats.** Quantitative metabolite profiles of gastrointestinal contents after oral administration of \([^{14}\text{C}]\text{CS-1036}\) (1 mg/kg) are shown in Fig. 5. As shown in Fig. 5A, radioactivity was mainly distributed in the ileum contents (67.7% of the dose) at 1 hour after the dose, in the ileum and cecal contents (33.9 and 49.9% of the dose, respectively) at 2 hours after the dose, and in the cecal contents (49.4–72.7% of the dose) after 4 hours after the dose. The radioactivity-time profiles of CS-1036 in each gastrointestinal section almost overlapped with those of the total radioactivity (Fig. 5B). M1 was below 1% of the dose in the stomach and intestinal contents at each time point, but it increased in a time-dependent manner in cecal contents to 7.1% of the dose at 8 hours after the dose (Fig. 5C). Although M2 was not observed in stomach or intestinal contents at any time points, small amounts of M2 (1.7–2.1%...
of the dose) were observed in cecal contents at 6 and 8 hours after the dose.

**Suppressive Effect on Plasma Glucose Elevation after Starch Loading in ZDF Rats.** As shown in Fig. 6, CS-1036 suppressed plasma glucose elevation after starch loading in ZDF rats in a dose-dependent manner. The plasma glucose elevations in the groups that were orally treated with CS-1036 at doses of 0.01, 0.03, and 0.1 mg/kg were significantly suppressed compared with the control group \( (P < 0.05) \). The ED\(_{50} \) was estimated to be 0.015 mg/kg according to the sigmoid E\( \text{max} \) model.

\[ R_0 \text{ of } [14C]CS-1036. \] The \( R_0 \) of \([14C]CS-1036 \) was 0.575–0.581 in rats, 0.595–0.603 in monkeys, and 0.521–0.538 in humans. No concentration dependency was observed in the range of 113–11300 nM.

**Discussion**

After intravenous administration of \([14C]CS-1036 \) to rats and monkeys, the radioactivity was mainly excreted via the urinary route (77.21% for rats and 81.13% for monkeys). These results suggest that the renal clearance is a major fraction of the CL of CS-1036. In both animals, the CL of CS-1036 was almost constant at the dose range of 0.3–1.0 mg/kg and the blood clearances of CS-1036 (CL/R\( _b \): 4.6–6.0 ml/min/kg for rats and 3.7–4.8 ml/min/kg for monkeys) were 6- to 8-fold lower than the renal blood flows (36.8 ml/min/kg for rats and 27.6 ml/min/kg for monkeys) (Davies and Morris, 1993). The \( V_{ss} \) of CS-1036 in rats and monkeys was also small and constant at the studied doses (0.218–0.237 l/kg for rats and 0.217–0.271 l/kg for monkeys), which was comparable with the extracellular fluid volume: 0.3 l/kg for rats and 0.2 l/kg for monkeys (Davies and Morris, 1993). Because the lipophilicity is reported to correlate positively with the \( V_{ss} \) (Poulin and Theil, 2002), the highly hydrophilic physicochemical property (estimated ClogP by Pallas: −4.57) of CS-1036 might lead to a low \( V_{ss} \).

After oral administration, the main route of excretion was feces (80.28% of the dose for rats, and 88.13% of the dose for monkeys), and the main component in feces was CS-1036 (70.3% of the dose up to 24 hours after the dose for rats, and 48.7% of the dose up to 48 hours after the dose for monkeys). On the other hand, the urinary excretion of CS-1036 was low in both animals (1.4% of the dose up to 24 hours after the dose for rats, and 2.9% of the dose up to 48 hours after the dose for monkeys), and was comparable to \( F_{\text{oral}} \) of both animals (1.73–2.44% for rats, and 0.983–1.20% for monkeys). These results suggest that most of the unabsorbed radioactivity is recovered from feces and that the absorbed radioactivity is mainly excreted into urine. The low \( F_{\text{oral}} \) of CS-1036 has a possibly strong relation to the highly hydrophilic trisaccharide structure of CS-1036. Kestose, which is also a trisaccharide compound as CS-1036, was reported to have no transmural potential in rats (Tsuiji et al., 1986).

The biliary excretion was negligible in BDC rats for both intravenous and oral administration (below 1% of the dose), although the fecal excretion (15.66% of the dose) after intravenous administration was much higher than the biliary excretion. This discrepancy may suggest the contribution of intestinal secretion to the fecal excretion of CS-1036. In some drugs, intestinal efflux transporters such as multidrug resistance 1 (MDR1), breast cancer resistance protein (BCRP), and multidrug resistance protein 2 (MRP2) are involved in the intestinal secretion (Lowes and Simmons, 2002; Haslam et al., 2011). However, the contribution of intestinal efflux transporters to the intestinal secretion of CS-1036 might be negligible because CS-1036 is more hydrophilic than the substrates of intestinal efflux transporters. Although the mechanism of intestinal secretion is unknown, we consider intestinal secretion of CS-1036 to be a specific phenomenon in rats because fecal excretion was below 1% of the dose after intravenous administration of \([14C]CS-1036 \) to monkeys.

After oral administration of \([14C]CS-1036, M1 and M2, the N- \] acetyl form of M1, were identified as metabolites in rat cecal contents and monkey feces, and only M1 was identified as a metabolite in rat and monkey urine. The in vitro metabolism study using hepatic, renal, and intestinal S9 fractions exhibited no metabolite observation after incubation of \([14C]CS-1036 \) in these S9 fractions, suggesting that hepatic, renal, and intestinal enzymes were not involved in the metabolite formation of CS-1036. M2 was observed in addition to M1 metabolic pathways of CS-1036 by intestinal flora are proposed in Fig. 7. As observed in CS-1036, acarbose, which is an \( a\)-GI and

**Fig. 6.** Change of plasma glucose level from predose (A) and AUC of change of plasma glucose level from baseline (B) after single oral administration of CS-1036 with starch (2 g/kg) to ZDF rats. The data are expressed as mean ± S.E. \((n = 8)\). The \( \Delta\text{AUC}_{\text{EC}} \) in the groups treated with starch and CS-1036 was compared with that of the starch control group by a Dunnett test. *\( P < 0.05 \) versus the starch control group.
oligosaccharide derivative, is also reported to be metabolized by intestinal flora, and the primary metabolites were formed by hydrolysis of O-glycosidic bonds in the molecule (Ahr et al., 1989). In addition to intestinal flora, α-glucosidase might be involved in the metabolism of acarbose because acarbose exhibits α-glycoside bonds in its molecule. For other α-GLIs, the hydrolysis of O-glycosidic bond by intestinal flora is not observed because the chemical structures of voglibose and miglitol are monosaccharide and do not exhibit O-glycosidic bonds in their molecules (Ahr et al., 1997; Hara and Hotta, 1997). For CS-1036, β-glucosidase from intestinal flora might be involved in the metabolism because CS-1036 exhibits a β-glycoside bond in its molecule and was stable in intestinal S9 fractions which contain α-glucosidase (Hauri et al., 1979; Goldin, 1990).

After oral administration of [14C]CS-1036, radioactivity and CS-1036 were rapidly eliminated from stomach, duodenum, and jejunum contents; the peak of CS-1036 in ileum contents was observed at 1 hour after the dose (66% of the dose), and most of the CS-1036 in the ileum contents was diminished at 4 hours after the dose. In ZDF rats, CS-1036 showed suppressive effects on the glucose elevation from 0.5 to 2.0 hours after oral administration of CS-1036 at ≈0.01 mg/kg (ED50 = 0.015 mg/kg). Alpha-amylase, which digests starch into oligosaccharides, is mainly distributed in the intestine in rats (McGeachin and Ford, 1959). Assuming the linear PK from the result of oral dosing in rats (0.3–10 mg/kg), the estimated intestinal concentration calculated from the intestinal amount of CS-1036 after an oral dose (0.01 mg/kg) and the intestinal fluid volume (McConnell et al., 2008) was estimated to be 1.5–3.1 μM from 0.5 to 2.0 hours after the dose, which will exceed the 50% inhibitory concentration (IC50 = 0.45 μM) to pancreatic amylase (Honda et al., 2004).

This suggests that CS-1036 exerts the suppressive effects on the glucose absorption when the concentration of CS-1036 in its site of action, intestinal lumen, is achieved to IC50 and over. Thus, the low absorption and a favorable stability in gastrointestinal contents of CS-1036 are considered to be its optimal properties as an α-amylase inhibitor. In addition to our estimation of intestinal CS-1036 concentration, some simulation tools might help us to predict the intestinal concentration in humans by taking into account the species differences in the length, transit time, and pH of each intestinal section (Parrott and Lave, 2002; Jamei et al., 2009). Not only the transition and concentration of CS-1036 but also the transitions of starch (which is the substrate of α-amylase) and α-amylase itself are important factors for the pharmacologic activity. Precise human pharmacokinetic/pharmacodynamic prediction is expected to be possible if the species differences of intestinal transition and activity of α-amylases secreted from saliva and pancreatic fluid are revealed.

M1 was reported as DAB, which is a potent inhibitor of hepatic glycogen phosphorylase (GP), with IC50 of 1.0 and 1.1 μM for inhibition to basal and glucagon-stimulated glycogenolysis in rat hepatocytes, respectively (Andersen et al., 1999). In ob/ob mice, the inhibition constant (Km) of DAB on GP was reported to be 392 nM (Fosgerau et al., 2000). Although the M1 (DAB) is reported to be highly absorbed (Foral 89%) (Mackay et al., 2003), the Cmax of M1 (<300 nM) after oral administration of CS-1036 at 10 mg/kg to rats was approximately 3/4 of the in vivo Km on GP in mice. On the other hand, the suppressive effect of CS-1036 on glucose elevation was observed at 0.01 mg/kg. These facts suggest that the pharmacologic effect of M1 via GP inhibition is almost negligible after CS-1036 treatment.

In conclusion, CS-1036 exhibited a low absorption, which leads to high distribution in its site of action, the intestinal lumen. Furthermore, most of CS-1036 was stable in the intestinal lumen, although a small amount of metabolites were produced by intestinal flora. The suppression of glucose absorption is suggested to be closely related to the transition of CS-1036 in the gastrointestinal system; therefore, CS-1036 showed a suppressive effect on postprandial hyperglycemia in rats.

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Authorship Contributions


Performed data analysis: Honda, Kaneno-Urasaki, Murai. Wrote or contributed to the writing of the manuscript: Honda, Murai, Nasu, Koga, Okuno, Izumi.

References


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