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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Pharmacokinetics of CS-1036 in rats and monkeys

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List of Abbreviations:

α-GI, α-glucosidase inhibitor; AUC, area under the plasma concentration versus time curve; AUC$_{\text{last}}$, area under the plasma concentration versus time curve up to the last quantifiable time; AUC$_{0-\text{inf}}$, area under the plasma concentration versus time curve up to infinity; AUMC, area under the first moment of the plasma concentration-time curve; ΔAUC$_{PG}$, area under the curve of change of plasma glucose level normalized by the plasma glucose level at 0 h as a baseline; ΔAUC$_{PG,\text{max}}$, area under the curve of change of plasma glucose level normalized by the plasma glucose level at 0 h as a baseline in the starch control group; ΔAUC$_{PG,\text{min}}$, area under the curve of change of plasma glucose level normalized by the plasma glucose level at 0 h as a baseline in the non-starch control group; BDC, bile duct-cannulated; CI, confidence intervals; CL, total body clearance; C$_{\text{max}}$, maximum plasma concentration; CS-1036, (2R,3R,4R)-4-hydroxy-2-(hydroxymethyl)pyrrolidin-3-yl 4-O-(6-deoxy-β-D-glucopyranosyl)-α-D-glucopyranoside; C$_{\text{tz}}$, last measurable concentration; DAB, 1,4-dideoxy-1,4-imino-D-arabinitol; F$_{\text{oral}}$, oral bioavailability; F344, F344/DuCrI Crj; γ, sigmoidicity factor; GP, glycogen phosphorylase; HPLC, high-performance liquid chromatography; IC$_{50}$, 50% inhibitory concentration; K$_i$, inhibition constant; λ, terminal elimination rate constant; LC, liquid chromatography;
MS, mass spectrometry; MS/MS, tandem mass spectrometry; PK, pharmacokinetics; 
PYF, peptone yeast extract Fildes solution; R_B, blood/plasma ratio; t_{max}, time to reach 
maximum plasma concentration; V_{ss}, volume of distribution at steady state; ZDF, 
Zucker diabetic fatty
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-D-glucopyranoside (CS-1036), a novel and potent pancreatic and salivary α-amylase inhibitor, were evaluated in F344/DuCrI Crlj 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 \([^{14}C]CS-1036\) 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 the fecal excretion after intravenous administration was observed (15.66%) and the 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 h post-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

\((2R,3R,4R)-4\text{-hydroxy}-2\text{-(hydroxymethyl)pyrrolidin-3-yl}\)

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

In this study, the absorption, metabolism, gastrointestinal distribution and excretion of CS-1036 were investigated in F344/DuCrlCrj (F344) rats and cynomolgus monkeys. To confirm the certainty of the assumed intestinal concentrations of CS-1036 affecting the inhibitory effects of CS-1036 on starch digestion, the suppression of plasma glucose elevation was investigated in Zucker diabetic fatty (ZDF, ZDF/Crl-Leprfa) rats after starch loading.
Materials and Methods

Materials.

CS-1036, and its metabolites M1, M2 and M3 were synthesized at Daiichi Sankyo Co., Ltd. (Tokyo, Japan) according to the published procedures (Honda et al., 2004). The internal standards, $^2$H$_5$-CS-1036 and $^2$H$_5$-M1 (Fig. 1), for the quantification of CS-1036 and M1 were also synthesized at Daiichi Sankyo Co., Ltd. $^{14}$C-CS-1036 (41.9, 52.2, 42.2 and 63.5 μCi/mg: 4 different lots) was synthesized at GE Healthcare UK Ltd. (Buckinghamshire, UK) and Sekisui Medical Co., Ltd. (Tokyo, Japan). The radiochemical purities of $^{14}$C-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 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 5 days and over. 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 supplied once daily to monkeys.
Water was given _ad libitum_ throughout the experiments. All the animals were fasted
overnight before administration and through to 4–8 h post-dose.

**Pharmacokinetics of CS-1036 in Rats and Monkeys.**

CS-1036 (0.3, 1, 3 and 10 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 h post-dose. Plasma was obtained by
centrifugation at 4°C and 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 h post-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 h post-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 administrated 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 h 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 h post-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 h and 48 h post-dose from rats and monkeys, respectively. Urine was applied to solid phase extraction using Oasis MCX cartridges (60 mg, 3 cc, Waters Corp.), 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 cc, 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.

$[^{14}C]$CS-1036 (final concentration: 22.7 µM, 0.42 µCi/ml) was incubated at 37°C for 1 h in 2 mg protein ml of hepatic, renal and intestinal S9 fractions from rats, monkeys and humans in 100 mM of potassium phosphate buffer (pH 7.4) with an NADPH-generating system (2.5 mM of β-NADP, 25 mM of glucose-6-phosphate, 0.5 units/ml of glucose-6-phosphate dehydrogenase, 10 mM of 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 F344 rats were sacrificed, the cecum was removed and the cecal contents were collected (n = 4). Feces were collected using a sterile swab from 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 monkey and human feces were suspended in 5 ml of peptone yeast extract Fildes solution (PYF) broth within 24 h of collection. The suspensions of rat cecal contents, and monkey and human feces were cultured individually at 35°C for 3 days in an incubator attached to an anaerobic chamber. Each 0.3 ml of the culture broth was transferred to 30 ml of PYF broth. The subcultured PYF broth was incubated at 35°C for 2 days in an incubator attached to the anaerobic chamber. Then, [14C]CS-1036 (final concentration: 22.7 µM, 63.5 µCi/ml) was incubated in PYF culture broths of rat cecal contents, and monkey and human feces at 35°C for 24 h under anaerobic conditions. The reaction was terminated by 3 cycles of freeze-thaw processes and then the same volume of acetonitrile was added. The supernatant (0.1 ml) obtained by centrifugation of the sample was dried up using a centrifugal evaporator, and the residues were reconstituted by 50 µl of water for analysis.

Sample Preparation for Quantitative Analysis of Radioactivity in Urine and Feces.

[14C]CS-1036 (1 mg/kg, 42.2 µCi/kg) was administered orally to fasted F344 rats
(164–168 g, n = 3). After administration, urine and feces were collected up to 24 h post-dose. 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. 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.

[^14C]CS-1036 (1 mg/kg, 42.4 μCi/kg) was administered orally to fasted F344 rats (148–168 g, n = 3). After administration, the stomach, duodenum and jejunum, ileum and cecum were observationally isolated, and each gastrointestinal content was collected at 0.5, 1, 2, 4, 6 and 8 h post-dose by washing with 5 ml of water three times (total: 15 ml). Each of the gastrointestinal content was homogenized, mixed with the same volume of methanol, and centrifuged (4°C, 1,600 g, 10 min). Then, the resulting supernatant was collected, and the residual extract was freeze-dried. The resulting residues from the stomach, duodenum and jejunum, and ileum contents were reconstituted by 50% methanol (0.2 ml), respectively. Each resulting solution was
centrifuged (4°C, 10,000 g, 10 min) to obtain the supernatant as an HPLC sample. As for the cecal contents, each resulting residue obtained by freeze-drying was mixed with 50% methanol (0.2 ml) and then centrifuged (4°C, 10,000 g, 10 min), and the resulting supernatant was collected. The extraction procedures were repeated 3 times. The three batches of supernatants were combined and evaporated under a nitrogen stream. The resulting residues from the cecal contents were dissolved in 50% methanol (0.2 ml), and each resulting solution was centrifuged (4°C, 10,000 g, 10 min) to obtain the supernatant as an HPLC sample.

**Effect of CS-1036 on Plasma Glucose Level after starch loading in ZDF rats.**

CS-1036 (0, 0.001, 0.003, 0.01, 0.03 and 0.1 mg/kg) in the starch suspensions (2 g/kg) was administered orally to fasted ZDF rats (300–366 g, n = 8 each). For the non-starch control, water (10 ml/kg) was administered to ZDF rats (314–361 g, n = 8). Blood was collected 15 min before administration and at 0.5, 1, 2 and 4 h post-dose. Plasma was obtained by centrifugation at room temperature and stored at 4°C until measurement of the plasma glucose levels. The plasma glucose level at 15 min before the administration was regarded as that at 0 h.
Blood/plasma Ratio ($R_B$) of $[^{14C}]$CS-1036.

Aliquots of 5 µl of $[^{14C}]$CS-1036 solution (final concentrations: 113, 227, 1130, 2270 and 11300 nM) were mixed with 495 µl of rat, monkey or human blood and incubated for 5 min at 37°C. The radioactivity of blood (0.1 ml) and plasma (0.1 ml), which was obtained by centrifugation at 4°C, was measured, and the $R_B$ was calculated by dividing blood radioactivity by plasma radioactivity.

Sample Analysis.

Quantification of CS-1036 and M1.

Plasma concentrations of CS-1036 and M1 were determined by LC-tandem mass spectrometry (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 $^2$H$_3$-CS-1036 and 100 ng/ml of $^2$H$_3$-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. Then, the sample was subjected to a solid-phase extraction (VersaPlate SCX, 100 mg, Agilent Technologies Inc., Santa Clara, CA, USA). The extracted sample was evaporated by nitrogen stream, and the residue was dissolved in 180 µl of mobile phase A (described below), and then 20 µl of the sample was
injected to an LC-MS/MS system consisting of an API4000 mass spectrometer (Applied Biosystems, Foster City, CA, USA) coupled to an HPLC system (Agilent 1100 series HPLC, Agilent Technologies Inc. or 2795 separations module, Waters Corp., Milford, MA, USA). The analytes were separated on a Capcell-pak UG80 SCX (5 μm, 2.0 × 75 mm; Shiseido Corp., Tokyo, Japan), at a column oven temperature of 40°C. The flow rate was 0.4 ml/min with a gradient of 10 mM ammonium acetate in methanol/water (50/50, v/v, A) and 10 mM ammonium acetate in methanol/water (95/5, v/v, B). The gradient condition of mobile phase B was 0% from zero to 4.5 min (constant), 0% to 100% for 4.5 to 5 min (linear), 100% for 5 to 5.5 min (constant) and 0% from 5.5 to 12 min (constant). Ionization was conducted in the positive ion electrospray ionization mode at a source temperature of 600°C, using nitrogen nebulizing and heating gas.

CS-1036 and its internal standard were analyzed in the multiple reaction monitoring mode using the mass transitions of \( m/z \ 442.4 \rightarrow 134.0 \) and \( 447.4 \rightarrow 139.0 \), respectively. M1 and its internal standard were analyzed in multiple reaction monitoring mode using the mass transitions of \( m/z \ 134.2 \rightarrow 68.1 \) and \( 139.2 \rightarrow 72.1 \), respectively.

**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., Buckinghamshire, UK or 1 ml of tissue solubilizer for BIOMERIT; Nakalai Tesque, Inc., Kyoto, Japan) and scintillation cocktail (10 ml of Hionic-Fluor; PerkinElmer Inc., Waltham, MA, USA or Cleasol I; Nacalai Tesque, Kyoto, Japan), and then analyzed with a liquid scintillation analyzer (Tri-carb 307, 2300TR or 2900TR counter; PerkinElmer, Inc.). Blood (0.1 ml) was mixed with 30% hydrogen peroxide (0.1 ml), a tissue solubilizer (1 ml of NCS-II) and a scintillation cocktail (10 ml of Hionic-Fluor), and then analyzed with a liquid scintillation analyzer. Feces were homogenized with 50–160 ml or 4-fold volume of water. Next, 0.5 ml of the fecal homogenate and gastrointestinal tract content homogenate was mixed with a tissue solubilizer (1 ml NCS-II or tissue solubilizer for BIOMERIT) and a scintillation cocktail (10 or 15 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 volumes of the sample weight and then homogenized. The fecal homogenate (0.5 ml) was placed on a combust pad, weighed and then combusted with a sample oxidizer. The resulting $^{14}$CO$_2$ was absorbed in Carbo-Sorb E (6 ml; PerkinElmer, Inc.), mixed with a scintillation cocktail (9 ml, Permafluor E+; PerkinElmer, Inc.), 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 coupled 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 L-2000 HPLC system (Hitachi High-Technologies Corp., Tokyo, Japan) equipped with a Radiomatic 625TR radioactivity detector (PerkinElmer, Inc.), and a mass spectrometer (Accela-LTQ Orbitrap XL; Thermo Fisher Scientific Inc., Waltham, MA, USA). 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 min (constant), 95% to 75% for 5 to 25 min (linear), 75% from 25 to 30 min; injection
volume, 10 μl; liquid scintillator for radioactivity detector, Ultima Flo M or Flo-Scint II (PerkinElmer Inc.); and scintillator flow rate, 3 ml/min. LC-MS conditions for qualitative metabolite analysis were as follows: ionization, positive ion electrospray ionization mode; capillary voltage, 3 kV; cone voltage, 40 V; collision energy, 10 eV; source temperature, 100°C; and desolvation gas temperature, 300°C. The spectral data were acquired in a mass range from m/z 50–1000. LC-MS conditions for quantitative metabolite analysis were as follows: ionization, positive ion electrospray ionization mode; spray voltage, 5 kV; capillary voltage, 27 V; and capillary temperature, 350°C.

**Plasma Glucose.**

The plasma glucose levels (mg/dL) were measured with an automatic glucose analyzer (Glucoroder-GXT, A&T Corp., Kanagawa, Japan).

**Pharmacokinetic Analysis.**

The PK parameters of CS-1036 and its metabolite M1 in plasma were calculated using WinNonlin Professional (Version 4.0.1; Pharsight Corp., Mountain View, CA, USA) based on a non-compartmental method. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were obtained by observed data. The t_{1/2} was
calculated by the regression analysis of 3 or more log-transformed data points in the terminal phase. The area under the plasma concentration versus time curve up to the last quantifiable time \( \text{AUC}_{\text{last}} \) was calculated by the trapezoidal method. AUC up to infinity \( \text{AUC}_{0-\text{inf}} \) was determined using the equation:

\[
\text{AUC}_{0-\text{inf}} = \text{AUC}_{\text{last}} + \frac{C_{tz}}{\lambda}.
\]

where, \( C_{tz} \) is the last measurable concentration and \( \lambda \) is the terminal elimination rate constant. For intravenous administration, the total body clearance (CL) was calculated as the ratio of dose and \( \text{AUC}_{0-\text{inf}} \). The volume of distribution at a steady state (\( V_{ss} \)) was calculated as the dose \( \cdot \text{AUMC}/(\text{AUC}_{0-\text{inf}})^2 \), where AUMC is the area under the first moment of the plasma concentration-time curve. \( F_{\text{oral}} \) for each dose was calculated by dividing \( \text{AUC}_{0-\text{inf}} \) for oral dose by that for intravenous dose at the same dose level.

**Evaluation of Plasma Glucose Levels.**

The changes of plasma glucose levels after the administration were calculated by subtracting the plasma glucose level at 0 h from that at each time point in the corresponding animal. The area under the curve of change in the plasma glucose level \( (\Delta \text{AUC}_{PG}) \), which was normalized by the plasma glucose level at 0 h as a baseline, was calculated by the trapezoidal method. The ED\(_{50}\) and its 95% confidence intervals (CI)
were estimated according to the sigmoid $E_{\text{max}}$ model described below, where the 
$\Delta \text{AUC}_{\text{PGS}}$ in the starch control group and the $\Delta \text{AUC}_{\text{PGS}}$ in the non-starch control group were substituted for the upper limit ($\Delta \text{AUC}_{\text{PG,\text{max}}}$) and lower limit ($\Delta \text{AUC}_{\text{PG,\text{min}}}$) 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, USA).

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

**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. $\Delta \text{AUC}_{\text{PGS}}$ in the CS-1036 treated groups were compared with those in the starch control group by a Dunnett test. $P$ values of less than 0.05 were considered to be 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 h for rats and 1.75 h for monkeys. The mean $F_{oral}$ was 1.73–2.44% for rats and 0.983–1.20% for monkeys.

Excretion of Radioactivity.

Excretion of radioactivity after intravenous and oral administration of
[\textsuperscript{14}C]CS-1036 to rats and monkeys is summarized in Table 3. After intravenous administration of \textsuperscript{14}C]CS-1036 to rats and monkeys, most of radioactivity was excreted into urine: 77.21% of the dose up to 168 h post-dose for rats and 81.13% of the dose up to 336 h post-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 h post-dose mainly as a result of unabsorbed radioactivity. In rats, the fecal excretion was 80.28% of the dose up to 168 h post-dose, in which the component of intestinal secretion might be included in addition to the unabsorbed radioactivity, because the fecal excretion was observed (15.66% of the dose) after intravenous administration and the biliary excretion in BDC rats was almost negligible (0.37% of the intravenous administration and 0.59% of the oral administration up to 48 h post-dose). 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 [$^{14}$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. Then, 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 $m/z$ at 176, and was consistent with its authentic standard. Then, M2 was identified as N-acetylated M1.

**In Vitro Metabolism of CS-1036.**

To identify if 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 [$^{14}$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 (data not shown). 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 monkey and human feces under anaerobic conditions. After 24 h of incubation, M1 was produced as a common metabolite of CS-1036 for rats, monkeys and humans (Fig. 4). M3 was observed only in the culture broth of rat cecal contents, and identified as N-acetyl metabolite of CS-1036 by following LC-MS/MS analysis.

Quantitative Plasma Profiles of M1 in Rats and Monkeys.

After oral administration of [14C]CS-1036 to rats and monkeys, metabolites were not observed in the radiochromatogram of the plasma samples due to the detection limit (data not shown), but M1 was observed in urinary samples. Therefore, M1 concentrations in plasma were determined 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 single intravenous administration of CS-1036, M1 was detected at low levels in rats at doses of 3 and 10 mg/kg. At the lower doses (0.3 and 1 mg/kg) in rats and at all dose levels (0.3–10 mg/kg) in monkeys, M1 was not detected. After single oral administration of CS-1036, the t_{max} of M1 was observed at 0.688–1.33 h post-dose in rats and at 1.25–3.5 h post-dose in monkeys. The
molar-based ratio of the AUC of M1 to that of CS-1036 ranged from 22.1–83.5% 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 h for rats and up to 48 h for monkeys after oral administration of [14C]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, species difference in the metabolite formation was observed, and 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 in the in vivo metabolite identification.

**Quantitative Metabolite Profiles of Gastrointestinal Contents in Rats.**

Quantitative metabolite profiles of gastrointestinal contents after oral administration of [14C]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 h post-dose, in the ileum and cecal contents (33.9% and 49.9% of the dose, respectively) at 2 h post-dose, and in the cecal contents (49.4%–72.7% of the dose) after 4 h.
post-dose. Radioactivity-time profiles of CS-1036 in each gastrointestinal section were almost overlapped with those of total radioactivity (Fig. 5B). M1 was below 1% of the dose in stomach and intestinal contents at each time point, but increased time-dependently in cecal contents to 7.1% of the dose at 8 h post-dose (Fig. 5C). Although M2 was not observed in stomach and intestinal contents at any time points, small amounts of M2 (1.7–2.1% of the dose) was observed in cecal contents at 6 and 8 h post-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 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$_{max}$ model.

$R_B$ of $^{14}$C-CS-1036.

$R_B$ of $^{14}$C-CS-1036 in rats, monkeys and humans 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, 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 mg/kg and the blood clearances of CS-1036 (CL/Rb; 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 Vss 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). Since the lipophilicity is reported to correlate positively with the Vss (Poulin and Theil, 2002), the highly hydrophilic physicochemical property (estimated ClogP by Pallas: –4.57) of CS-1036 might lead to a low Vss.

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 h post-dose for rats and 48.7% of the dose up to 48 h post-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 h post-dose for rats and 2.9% of the dose up to 48 h post-dose for monkeys), and was comparable to $F_{oral}$ 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 the absorbed radioactivity is mainly excreted into urine. The low $F_{oral}$ 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 (Tsuji et al., 1986). The biliary excretion was negligible in BDC rats for both intravenous and oral administration (below 1% of the dose), although the 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 MDR1, BCRP and MRP2 involved in the intestinal secretion (Lowes and Simmons, 2002; Haslam et al., 2011). However, the contribution of intestinal efflux transporters on 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 was unknown, intestinal secretion of CS-1036 was considered as a specific phenomenon in rats because the 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, 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 in feces, and M1 in plasma was higher after oral administration than intravenous administration to rats and monkeys. Furthermore, M1 was produced in a culture broth of rat cecal contents, monkey feces and human feces under anaerobic conditions. M2 was not observed, but M3, N-acetyl form of CS-1036, was identified in the culture broth of rat cecal contents. Intestinal flora is reported to have the potential for N-acetylation in humans, rats, mice and dogs (King et al., 1990; Okumura et al., 1995). These facts suggest that CS-1036 metabolized by enzymes of intestinal flora. In addition, M1 and M2 were higher in monkey feces than in rat feces, indicating that the metabolite formation of CS-1036 by intestinal flora was higher in monkeys than in rats.
Based on the \textit{in vivo} and \textit{in vitro} metabolite information, the metabolic pathways of CS-1036 by intestinal flora are proposed in Fig. 7. As observed in CS-1036, acarbose, which is an $\alpha$-GI and 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, $\alpha$-glucosidase might be involved in the metabolism of acarbose because acarbose exhibits $\alpha$-glycoside bonds in its molecule. For other $\alpha$-GIs, 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, $\beta$-glucosidase from intestinal flora might be involved in the metabolism because CS-1036 exhibits a $\beta$-glycoside bond in its molecule and was stable in intestinal S9 fractions which contain $\alpha$-glucosidase (Hauri et al., 1979; Goldin, 1990).

After oral administration of [$^{14}$C]CS-1036, radioactivity and CS-1036 was rapidly eliminated from stomach, duodenum and jejunum contents, and the peak of CS-1036 in ileum contents was observed at 1 h post-dose (66% of the dose) and most of CS-1036 in ileum contents was diminished at 4 h post-dose. In ZDF rats, CS-1036 showed the suppressive effects on the glucose elevation from 0.5 to 2 h after oral administration of
CS-1036 at 0.01 mg/kg and over and ED50 at 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 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 h post-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 the optimal properties as an α-amylase inhibitor. In addition to the estimation of intestinal CS-1036 concentration performed above, some simulation tools might help us to predict the intestinal concentration in humans by taking into account the species differences on 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 pharmacological activity. The precise human PK/PD
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 IC$_{50}$ 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 (K$_i$) of DAB on GP was reported to be 392 nM (Fosgerau et al., 2000). Although the M1 (DAB) is reported to be highly absorbed (F$_{oral}$; 89%) (Mackay et al., 2003), the C$_{max}$ of M1 (<300 nM) after oral administration of CS-1036 at 10 mg/kg to rats was approximately three-fourth of in vivo Ki 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 pharmacological 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 with the transition of CS-1036 in gastrointestinal, therefore, CS-1036 showed a suppressive effect on postprandial hyperglycemia in rats.
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We would like to acknowledge Takeshi Honda for the supply of compounds and Keiichi Fusegawa for his assistance with the animal experiments.
Authorship Contributions

Participated in research design: Honda, Murai, Okuno, Nasu, Koga, and Izumi.

Conducted experiments: Honda, Kaneno-Urasaki, Murai, Okuno, Kakuta, Nasu, and Namba.

Contributed new reagents or analytic tools: Honda, Kaneno-Urasaki, Murai, and Nasu.

Performed data analysis: Honda, Kaneno-Urasaki, and Murai.

Wrote or contributed to the writing of the manuscript: Honda, Murai, Nasu, Koga, Okuno, and Izumi.
References


Footnotes

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E-mail: honda.tomohiro.us@daiichisankyo.co.jp
Figure Legends

Fig. 1
Chemical structures of $^{14}$C]CS-1036 (A) and internal standard for CS-1036 (B) and internal standard for M1 (C).

Fig. 2
Plasma concentration profiles of CS-1036 after single intravenous and oral administration of CS-1036 to rats and monkeys at a dose of 10 mg/kg. The data are expressed as the mean + S.D. (n = 3 or 4).

Fig. 3
Representative radiochromatograms of the metabolites in rat and monkey urine and feces after oral administration of $^{14}$C]CS-1036.

Fig. 4
Representative radiochromatograms obtained by analysis of the culture broths of rat cecal contents, monkey feces and human feces after incubation of $^{14}$C]CS-1036 under
anaerobic conditions for 24 h.

**Fig. 5**

Total radioactivity, CS-1036 and M1 time-profiles in stomach, duodenum and jejunum, ileum and cecal contents after single oral administration of $[^{14}\text{C}]$CS-1036 to rats. The data are expressed as the mean ± S.D. (n = 3).

**Fig. 6**

Change of plasma glucose level from pre-dose (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 the mean ± S.E. (n = 8). The $\Delta$AUCPGs in the groups treated with starch and CS-1036 were compared with those in the starch control group by a Dunnett test. *: $P < 0.05$ vs. the starch control group.

**Fig. 7**

Proposed metabolic pathway of CS-1036.
<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>AUC_{last} (ng·h/ml)</th>
<th>AUC_{0-inf} (ng·h/ml)</th>
<th>CL (ml/min/kg)</th>
<th>V_{ss} (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>18700 ± 1300</td>
<td>18800 ± 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>53000 ± 5600</td>
<td>53200 ± 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>18100 ± 800</td>
<td>18200 ± 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>74300 ± 7300</td>
<td>74500 ± 7300</td>
<td>2.25 ± 0.22</td>
<td>0.217 ± 0.019</td>
<td>30.0 ± 4.6</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D. (n = 4).
Table 2   PK parameters of CS-1036 after single oral administration of CS-1036 to rats and monkeys

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>AUC_&lt;sub&gt;last&lt;/sub&gt; (ng·h/ml)</th>
<th>AUC_&lt;sub&gt;0-inf&lt;/sub&gt; (ng·h/ml)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>F&lt;sub&gt;oral&lt;/sub&gt; (%)</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>152 ± 77</td>
<td>12.6 ± 5.0</td>
<td>1.20</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D. (n = 3 or 4). -: not applicable.
Table 3  Excretion of radioactivity after single intravenous and oral administration of \([1^4\text{C}]\text{CS-1036}\) to rats and monkeys

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose/Route</th>
<th>Collection Period</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>86.96 ± 5.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0–168</td>
<td>77.21 ± 7.62</td>
<td>17.82 ± 8.83</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>95.85 ± 4.60</td>
</tr>
<tr>
<td></td>
<td>1 mg/kg, p.o.</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>93.26 ± 1.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0–168</td>
<td>15.32 ± 3.09</td>
<td>80.28 ± 3.88</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>97.94 ± 0.26</td>
</tr>
<tr>
<td>BDC rat</td>
<td>1 mg/kg, i.v.</td>
<td>0–48</td>
<td>-</td>
<td>-</td>
<td>0.37 ± 0.08</td>
<td>-</td>
<td>-</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0–48</td>
<td>-</td>
<td>-</td>
<td>0.59 ± 0.04</td>
<td>-</td>
<td>-</td>
<td>0.59 ± 0.04</td>
</tr>
<tr>
<td>Monkey</td>
<td>3 mg/kg, i.v.</td>
<td>0–48</td>
<td>80.13 ± 4.76</td>
<td>0.46 ± 0.16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13.70 ± 4.19</td>
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<tr>
<td></td>
<td></td>
<td>0–336</td>
<td>81.13 ± 4.92</td>
<td>0.66 ± 0.18</td>
<td>-</td>
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<td>-</td>
<td>14.50 ± 4.04</td>
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<tr>
<td></td>
<td>3 mg/kg, p.o.</td>
<td>0–48</td>
<td>4.54 ± 2.23</td>
<td>78.50 ± 8.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.76 ± 0.37</td>
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<tr>
<td></td>
<td></td>
<td>0–336</td>
<td>5.67 ± 2.33</td>
<td>88.13 ± 3.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.35 ± 0.79</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D. (n = 4). - : not tested.
Table 4  PK parameters of M1 after single intravenous and oral administration of CS-1036 to rats and monkeys

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Dose</th>
<th>t&lt;sub&gt;max&lt;/sub&gt;</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;last&lt;/sub&gt;</th>
<th>Ratio of molar-based AUC&lt;sub&gt;last&lt;/sub&gt; % of CS-1036</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/kg</td>
<td>h</td>
<td>ng/ml</td>
<td>ng·h/ml</td>
<td></td>
</tr>
<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>
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<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>
</tr>
<tr>
<td></td>
<td>p.o.</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>
</tr>
<tr>
<td></td>
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<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>
</tr>
<tr>
<td></td>
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<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>
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<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>
</tr>
<tr>
<td>Monkey</td>
<td>p.o.</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>
</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>
</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>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D. (n = 3 or 4) for rats and monkeys.
Table 5   Composition of radioactive components in urine and feces after single oral administration of [14C]CS-1036 to rats and monkeys

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Collection</th>
<th>Component</th>
<th>Excretion of Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>1 mg/kg</td>
<td>24</td>
<td>CS-1036</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M1</td>
<td>5.3 ± 1.3</td>
</tr>
<tr>
<td>Monkey</td>
<td>3 mg/kg</td>
<td>48</td>
<td>CS-1036</td>
<td>2.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M1</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M2</td>
<td>ND</td>
</tr>
</tbody>
</table>

Ratio of CS-1036 and its metabolites were determined by the ratio of excreted radioactivity and the peak area ratio of each component on the radiochromatogram. Values are expressed as the mean ± S.D. (n = 3). ND: not determined.
Fig. 1

(A) 

(B) 

(C)
Fig. 2
Fig. 3

Radioactivity (cpm)

Retention time (min)
Fig. 4

Radioactivity (cpm)

Retention time (min)

Rat cecal contents

Monkey feces

Human feces
Fig. 5
Fig. 6

(A) Change of plasma glucose level (mg/dl) over time (h) for different doses of the drug. The graph shows the following:
- Non-starch control
- Starch control
- 0.001 mg/kg
- 0.003 mg/kg
- 0.01 mg/kg
- 0.03 mg/kg
- 0.1 mg/kg

The ED50 is 0.015 mg/kg with a $\gamma$ value of -0.8.

(B) The graph shows the changes in $\Delta U_{AC}$ (mg-h/dl) with respect to dose (mg/kg): Starch control, 0.001 mg/kg, 0.01 mg/kg, 0.1 mg/kg, and Non-starch control. The $\Delta U_{AC}$ decreases with increasing dose.
Fig. 7