Cross-Species Differences in the Preclinical Pharmacokinetics of CT7758, an $\alpha_4\beta_1/\alpha_4\beta_7$ Integrin Antagonist

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ABBREVIATION LIST: BNPP, Bis(p-nitrophenyl)phosphate sodium; CL<sub>H</sub>, in vivo hepatic clearance; CL<sub>int</sub>, intrinsic clearance; Q<sub>H</sub>, hepatic blood flow; HBSS, Hank’s Balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LPAM, lymphocyte Peyer’s patch cellular adhesion molecule; MAdCAM, Mucosal addressin cell adhesion molecule; MES, (N-morpholino)ethanesulfonic acid; MIDAS, Mmetal-ion-dependent adhesion site of the integrinass of protonated molecule; MRM, multiple reaction monitoring; PSA, polar surface area; TEER, Transepithelial electric resistance; TFA, trifluoroacetic acid; VCAM-1, vascular adhesion molecule-1; VLA-4, very late antigen-4
ABSTRACT

CT7758, a carboxylate containing α4β1/α4/β7 integrin antagonist, was characterized for its pharmacokinetic profile in various in vitro and in vivo assays in support of clinical development. The oral bioavailability of CT7758 was 4 % in mice, 2 % in rats, 7-55 % in dogs, and 0.2 % in cynomolgus monkeys. The low bioavailability in rodents and monkey results from low intestinal absorption as evidenced by a low fraction absorbed in the rat portal vein model (3 %), low-to-medium permeability in Caco-2 cells (≤ 1.3x10^-6 cm/sec) with evidences of polarized efflux, and high polar surface area (104 Å). In rodents and cynomolgus monkeys, the total plasma clearance was moderate to high (≥ 50 % hepatic blood flow Qh) and associated with a short elimination half-life (≤1 h). This contrast with the dog data which showed a much lower clearance (6 % Qh) and a longer t1/2 (2.4 h). The volume of distribution (Vz) also varied significantly across species with value of 5.5, 2.8, 0.24 and 0.93 L/kg in mouse, rat, dog and cynomolgus monkey, respectively. In vitro assays demonstrated that active hepatic uptake accounted for most of the in vivo clearance and was the source of the large species variability. In vitro uptake assays predicted a total plasma clearance in humans in the low range (33 % Qh), a finding subsequently confirmed in the clinic. Assays in OAPT1B1-transfected cells demonstrated active uptake transport through this transporter. The prospect of limited absorption in human prompted the synthesis an ethyl ester prodrug, CDP323, which demonstrated higher in vitro permeability, increased oral bioavailability as well as efficient in vivo release of its active moiety CT7758.
INTRODUCTION

The α4 integrins are known to play a significant role in the recruitment and activation of leukocytes in inflammation and autoimmune diseases.

Most leukocytes including eosinophils, basophils, and monocytes, but not circulating neutrophils, constitutively express α4β1 integrin (very late antigen-4, VLA-4, CD49d/CD29). Its ligand is vascular cell adhesion molecule-1 (VCAM-1, CD106) which is expressed on endothelial cells and which is up-regulated in response to pro-inflammatory cytokines and chemokines. The interaction of α4β1 with VCAM-1 enables the rolling and the adhesion of leukocytes to the endothelium which is followed by their transendothelial migration through the vessel wall, proliferation and subsequently activation (Yang and Hagmann, 2003). This α4β1-mediated cell influx is involved in diseases such as asthma, rheumatoid arthritis, and multiple sclerosis.

The closely related integrin α4β7 integrin (LPAM, lymphocyte Peyer’s patch cellular adhesion molecule) is expressed on a more limited subset of leukocytes (macrophages, subset of lymphocytes, NK cells, mast cells, eosinophils). α4β7 binds to MAdCAM-1 (mucosal addressin cell adhesion molecule), which is expressed locally in gut mucosal tissues. MAdCAM-1 is up-regulated in the gut during inflammation and its interaction with α4β7 ensures the infiltration of leukocytes through the intestinal endothelium. Such cell trafficking is thought to play a pivotal role in ulcerative colitis and Crohn’s disease.

Since more than a decade, there have been many efforts to design therapies to antagonize α4 integrins to treat inflammatory diseases (Cox et al., 2010;Tilley, 2008). Natalizumab (Tysabri; Elan/Biogen-Idec), a humanized monoclonal anti-α4 antibody, has been approved for the
treatment of multiple sclerosis (Horga and de la Parte, 2007) and Crohn’s disease (Bickston and Muniyappa, 2010; Sands et al., 2009). Although natalizumab confirmed the clinical relevance of VLA-4 as a therapeutic target for these diseases, its usage is partially hampered by reports of progressive multifocal encephalopathy, a potentially fatal side effect.

Since mid 1990s, considerable efforts have been made to design small molecule VLA-4 antagonists with the advantages of reduced cost of goods, more convenient dosing regimen and possibly improved efficacy and/or safety. Most of the efforts have been concentrated around two scaffolds, Leu-Asp-Val (LDV) mimetics and N-acylphenylalanine derivatives. A common key structural element of both pharmacophores is a carboxylic acid group that is key for the binding to the Mg\(^{2+}\) of the metal-ion-dependent adhesion site of the integrin (MIDAS) (Takagi, 2007). Removing this carboxylic group invariably reduced the potency of the antagonist (Gong et al., 2008). Unfortunately, this ionized carboxylate group combined with high molecular weight, high hydrogen bound count have been responsible for poor intestinal absorption and/or rapid clearance (Davenport and Munday, 2007). In addition, active efflux and uptake transporters have been shown to play a major role in the poor pharmacokinetic properties (Tang et al., 2008). Such unfavourable pharmacokinetic liabilities were a major hurdle in the development of small molecule VLA-4 antagonists. The present work was carried out to document the preclinical pharmacokinetics of the phenylalanine-based VLA-4 antagonist CT7758 (Fig.1) (Davenport and Munday, 2007). Understanding the pharmacokinetics of drug molecule is fundamental in determining whether a compound should be progressed into the clinic. Increasingly, human pharmacokinetics could be predicted from data obtained in preclinical species. The disposition of CT7758 was fully investigated in vitro and in vivo and demonstrated complex pharmacokinetic pathways including active uptake and efflux transport, unpredictable absorption and large inter-species differences in total plasma clearance. In order to circumvent potential intestinal absorption
issues, the ethyl ester prodrug CDP323 (see structure in Fig.1) was synthesized. Preliminary
\textit{in vitro} and \textit{in vivo} pharmacokinetic investigations confirmed the improved intestinal
permeability of CDP323 while ensuring efficient \textit{in vivo} release of the active moiety, CT7758.
MATERIALS AND METHODS

Chemicals and Reagents

Bis (p-nitrophenyl)phosphate sodium (BNPP), sodium cholate and deoxycholate, MK571 and silicon oil were purchased from Sigma (Bornem, Belgium). [14C]-CT7758 and [14C]-CDP323 (50 mCi/mmol, >99 % radiochemical purity) were synthesized by Selcia (Essex, UK). CT7758 and CDP323 were synthetized at UCB S.A. All the other chemicals were of analytical grade and were purchased from commercial sources.

Animals

Male Wistar rats (250-320 g) and CD-1 mice (20-25 g) were supplied from Charles River Laboratories (Lyon, L’Arbresle, France). Non naïve male Beagle dogs weighing 10 kg were supplied by Marshall Farms (North Rose, New York, USA) and non-naïve male Cynomolgus monkeys weighing 3kg were supplied by Guangxi Xiongsen (China). Animals were acclimatized to study conditions before drug dosing. Pharmacokinetic studies in rodents were performed at internally at UCB S.A. while non-rodent studies were conducted at the CiToxLAB (Centre International de Toxicologie, Evreux, France). All animal studies were reviewed and approved by the local Animal Ethical Committees.

Pharmacokinetic Studies in Mouse, Rat, Dog and Cynomolgus Monkey

For plasma pharmacokinetic studies, CT7758 was administered orally (5-10 mL/kg, 30 mg/kg) or intravenously (2 mL/kg, 3 mg/kg) as a solution in 10mM phosphate buffer. CDP323 was administered orally as a 1 % methylcellulose suspension containing 0.1 % tween 80 (same dosage volume as CT7758). Compounds were delivered to fasted animals with the food returned 4h post-dose. Blood samples were collected at the designated time points. Plasma was prepared by centrifugation, collected and stored at -20°C until analysis by LC-
MS/MS. When CDP323 was administered, blood samples were collected into pre-chilled tubes pre-filled with 25 µL of a 15 mg/mL BNPP solution to prevent drug hydrolysis. For non-rodent studies, composite PK profiles were obtained. For the mouse study (3 mice/timepoint), terminal blood sampling was collected under isoflurane anesthesia from vena cava after laparotomy, and for the rat study (3 rats/timepoints), non-serial blood samples were collected from the retro-orbital sinus under isoflurane anesthesia for the first 2 timepoints and by cardiac puncture for the last sampling time. In dogs (n=3) and monkey (n=3), blood samples were collected from jugular vein (dog) or cephalic vein (monkey) of unanesthetized manually restrained animals.

Bile was collected in double catheterized free-moving male rats (n=3). Briefly, animals were anesthetized using inhaled isoflurane (1.5-3 %, flow of 1.5 L/min) and equipped with permanent polyurethane catheters (OD 0.41 mm, ID 0.23 mm, Instech Solomon, Plymouth Meeting, PA) implanted into the bile duct (in the liver direction on 2.5 mm) and the duodenum (at the level of the Oddi sphincter). The two catheters were connected to each other during the operation session to maintain an intact enterohepatic circulation. Ten days after the surgery, the enterohepatic circulation was interrupted and the natural bile was collected by connecting the collection bile catheter to a refrigerated vial. The supplementation of bile salt was ensured by infusion (flow of 0.6 mL/h) of artificial bile salts solution (sodium cholate/sodium deoxycholate [1/1]) through the catheter implanted into the duodenum. After recovery, animals received a single intravenous dose of [14C]-CT7758 (2 mL/kg) at 3 mg/kg (50 µCi/kg). Blood samples were collected manually from a catheter implanted in the vena cava.

In the in vivo pharmacokinetic study to assess the fraction absorbed in rats, CT7758 was administered following single oral (10 mL/kg) and intravenous dosing (2 mL/kg) at 30 mg/kg.
and 3 mg/kg (n=3/timepoints), respectively and terminal blood samples (1 mL/sampling site) were collected, under isoflurane anesthesia, simultaneously from portal vein and vena cava.

**LC-MS/MS analysis of CT7758 and CDP323 in plasma samples**

Plasma samples were analysed using a validated LC-MS/MS method. Samples were mixed with internal standards (hepta-deuterated analogues of CDP323 and CT7758) and loaded onto Oasis HLB 30 mg solid phase extraction plates (Waters Inc, Milford, MA). After loading, wells were washed and eluted. The extracts were then evaporated and reconstituted with the aqueous mobile phase. Analytes and internal standard were separated by reversed phase chromatography in gradient mode using a Polaris C18-A (3.0 µm, 50x2.0 mm) column (Agilent, Palo Alto, CA) equipped with a pre-column. Mobile phases consisted of a mixture of 5 % acetonitrile and 95 % of water containing 0.1 % of trifluoroacetic acid (TFA) at pH 2.0 for the aqueous mobile phase and 95 % acetonitrile and 5 % of water containing 0.1 % of TFA at pH 2.0 for the organic mobile phase. Flow rate was 0.7 ml/min. Under the selected gradient conditions retention times were 7.9 min for CT7758 and 8.8 min for CDP323. Detection was performed by electrospray mass spectrometry (Quattro Ultima, Waters, Milford, MA) using multiple reaction monitoring (MRM) in positive mode. The MRM transitions were m/z 549 to 347 and m/z 521 to 413 for CDP323 and CT7758, respectively. For the respective internal standards m/z 556 to 354 and m/z 528 to 420 were monitored. Cone voltage was set to 40 eV and collision energy to 25 eV for all analytes and internal standard. The method was formally validated (Shah et al., 1991) with analytical range of 1.00 to 500 ng/mL for CDP323 and 1.00 to 2500 ng/mL for CT7758.

**Caco-2 assay**
Caco-2 cells (HTB37, American Type Culture Collection, Rockville, MA, USA) were used at passage number 27-35. Cells were grown on microporous membranes (collagen I precoated culture inserts of polyethylene terephthalate) of 1.0 µm pore size (12-well plate) for 20-22 days in a 10 % CO₂; 90 % air atmosphere at 37 °C in classical Caco-2 culture medium (Hubatsch et al., 2007). Culture medium was also replaced 24 h before initiating the transport study.

For the transport experiment, the medium was discarded and replaced with Hank’s Balanced Salt Solution (HBSS) supplemented with 25 mM D-glucose, 1.25 mM calcium chloride and 0.5 mM magnesium chloride and referred to as transport medium. In addition, depending on the final pH (pH 5.0, 6.0, 6.5 and 7.4 for the apical side and pH 7.4 for the basolateral side), transport medium was supplemented with 20 mM (N-morpholino)ethanesulfonic acid (MES, pH 5.0, 6.0 and 6.5) or 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 7.4). The integrity of the monolayers was determined by the measurement of the transepithelial electric resistance (TEER) using an Epithelial VoltOhmmeter (World Precision Instruments, Stevenage, UK) just prior starting the assay. The assay was initiated by addition of [¹⁴C]-CDP323 or [¹⁴C]-CT7758 at the indicated final concentrations.

At each pre-determined incubation time, 100 µL (for apical to basolateral transport) or 50 µL (for basolateral to apical transport) of transport medium was sampled in the receiver compartment and replaced with the same volume of fresh transport medium and further incubated at 37°C under stirring conditions. Samples were mixed with 2 mL Ultima Gold scintillant (Perkin Elmer, Waltham, USA). The concentration of radiolabelled test compound was evaluated using a TRI CARB liquid scintillation counter (Perkin Elmer, Waltham, USA).

**Uptake by Cells expressing OATP1B1**

HEK293 cells expressing human OATP1B1 (hOATP1B1, OATP-C) as well as mock cells were obtained from Genomembrane®. Cells were routinely grown in Dulbecco’s modified
Eagle’s medium containing 10% fetal bovine serum, 100 IU/mL penicillin, 100 µg/mL streptomycin, in a humidified incubator at 37 °C under 5% CO₂. Before the uptake measurement, HEK293 cells were seeded in 12-well plates, coated with poly-d-lysine, at a density of 5×10⁵ cells/well. Cells were incubated overnight. The culture medium was discarded and replaced with 1mL of uptake medium (125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM CaCl₂, 1.2 mM MgSO₄, 1.2 KH₂PO₄ and 25 mM HEPES adjusted to pH 7.4). After a 5 min pre-incubation at 37 °C, the uptake experiment was initiated by replacement of uptake medium with pre-warmed fresh medium containing CT7758 or the reference OATP1B1 substrate [³H]-estrone-3-sulfate. At the end of incubation, cells were rinsed, collected in 500 µL of uptake medium and then sonicated to obtain cell lysates. CT7758 concentration in cell lysates were measured by LC-MS/MS analysis and [³H]-estrone-3-sulfate by liquid scintillation.

**In vitro hepatocytes assays**

Rat and mouse hepatocytes were prepared using a modification of Seglen’s two step collagenase perfusion technique (Seglen, 1976). Fresh Beagle dog and human hepatocyte preparations were obtained from Biopredic International (Rennes, France).

For metabolic clearance experiments, hepatocytes were suspended at 0.5 x10⁶ viable cells/mL in Williams' E medium without phenol red containing Glutamax-I, penicillin, streptomycin, bovine insulin and hydrocortisone hemissuccinate. Incubations (1 mL each) were initiated by the addition of [¹⁴C]-CDP323 or [¹⁴C]-CT7758 (5 µM and 0.3 µCi/mL final concentrations). The cells were then incubated in CO₂ incubator for 30, 60 and 180 min at 37°C under gentle agitation. At the end of the incubation period, the reaction was stopped by the addition of 1 mL ice-cold acetonitrile. The tubes were then thoroughly mixed, centrifuged at 10000 g for
10 min at 4°C, the supernatants were immediately collected and analyzed by radio-HPLC in order to measure parent drug and its related metabolite(s).

For uptake experiments, hepatocytes were suspended in HBSS at 1 x 10^6 viable cells/mL. The uptake was initiated by addition of [14C]-CT7758 to the suspension. At designated time points, the reaction was stopped by centrifuging the cells through a silicon oil/mineral oil layer (final density of 1.027) into 200 µL 2 N sodium hydroxide. The tube was directly thrown in nitrogen liquid and cut to collect the bottom part (containing the digested hepatocytes). An aliquot of cell digest was mixed with Ultima Gold scintillant and the radioactivity of the content was determined using a TRI CARB scintillation counter. Finally, protein determination was performed using bicinchoninic acid assay.

**Plasma Protein Binding**

The plasma protein binding of [14C]-CT7758 was determined in vitro using ultrafiltration technique. Briefly, plasma samples from mouse, rat, dog and human were spiked with CT7758 (cold and radiolabelled) to reach concentration of 0.3, 1, 3, 10 and 30 µg/mL. After mixing, plasma samples were incubated (under agitation) in a water bath at 37°C for 10 min. Then, a samples was then taken and centrifuged at approximately 1800g for 15 min using ultrafiltration apparatus (Centrifree Micropartition System, Amico Inc, MA, USA). Unfiltered plasma and ultrafiltrate were assayed for radioactivity content and free fraction was calculated as radioactivity content ratio of ultrafiltrate over unfiltered plasma.

**Radio-HPLC analysis of in vitro samples from metabolic stability assay**

For metabolic stability assay, samples were analysed by radio-HPLC. The HPLC system used was an Agilent 1100 series (Agilent Technologies, Palo Alto, USA) coupled with a Packard Radiomatic 515 TR radiochemical detector (Packard Instruments, Meriden, USA). The column was an Atlantis T3 (250 x 4.6 mm – 5 µm) (Waters, Torrance, USA) protected by a guard column Atlantis T3 (20 x 4.6 mm – 5 µm). The flow was adjusted to 1 mL.min⁻¹ at 30
\[ P_{\text{app}} (\text{cm/s}) = \left( \frac{dc}{dt} \right) \left( \frac{V_r}{A} \right) \left( \frac{1}{3600} \right) C_i \]

where \( dc/dt \) = the slope of cumulative receiver concentration versus time plot (dpm/mL/h) (the slope was calculated from the linear part of the curve);

\[ V_r = \text{the volume of Transport Medium in the receiver compartment (mL)}; \]

\[ A = \text{the surface area of monolayer (0.9 cm}^2); \]

\[ C_i = \text{the initial measured concentration in donor compartment (dpm/mL)}. \]

For pharmacokinetic analysis, individual plasma concentrations (for dog and monkey) or mean plasma concentrations (for rodents), nominal sampling times and nominal dose levels were used to compute CDP323 and CT7758 pharmacokinetic parameters (non-compartmental
For *in vitro* studies, the intrinsic metabolic clearance $\text{CL}_{\text{int,vitro}}$ describing parent drug consumption in hepatocytes was determined using the following equation:

$$ CL_{\text{int,vitro}} = -\frac{k}{[\text{Hep}]} $$

where $k$ is the slope obtained from non-linear regression of parent compound concentration in relation with incubation time (WinNonlin Software, version 5.0.1, Pharsight Corp., Mountain View, California 94040), and is equal to $0.693/t_{1/2}$.

$[\text{Hep}]$ is the hepatocyte density in the incubates ($10^6$ hep/mL)

These *in vitro* $\text{CL}_{\text{int,vitro}}$ values were used to calculate $\text{CL}_{\text{int,vivo}}$ (mL/min/kg) using the following scaling factors: i) $125 \times 10^6$ hepatocytes/g liver for all species except for dog where $240 \times 10^6$ hepatocytes/g liver was used, ii) liver weight of 87.5, 40, 32 and 23 g/kg for mouse, rat, dog and human, respectively, iii) hepatic blood flow ($Q_H$) of 90, 90, 30.9 and 22 ml/min/kg for mouse, rat, dog and human, respectively. Blood-to-plasma ratio ($R_{BP}$) was 0.8, 0.6, 0.44 and 0.6 in mouse, rat, dog and human, respectively. The free fraction in plasma ($fu$) was 0.01 in rat, dog and human while it was 0.024 in mouse and were used for the hepatic clearance prediction. As CT7758 is hydrophilic (Log D of 0.74), non-specific binding to hepatocytes was assumed to be negligible (predicted $fu_{hep}$ of 0.94) (Kilford et al., 2008).

The predicted hepatic clearance $\text{CL}_{H}$ was calculated using the well-stirred model:

$$ \text{CL}_{H} = \frac{Q_H \times R_{BP} \times fu \times \text{CL}_{\text{int,vivo}}}{Q_H \times R_{BP} + fu \times \text{CL}_{\text{int,vivo}}} $$
The uptake clearance was determined using the following equation:

\[
CL_{\text{int, uptake}} = \frac{v}{S}
\]

where \( v \) is the initial rate of uptake (linear with time and corresponding to the slope of the linear regression of the amount of compound uptake in relation to time)

\( S \) is the substrate concentration at time 0

\( CL_{\text{int, uptake}} \) was scaled up as for \( CL_{\text{int, vitro}} \).
RESULTS

3.1. In vivo Pharmacokinetics of CT7758

The pharmacokinetic profile of the carboxylic acid, CT7758 was investigated in various animal species after single iv (3 mg/kg) and oral administration (30 mg/kg). Mean plasma concentration time courses are shown in Fig. 2 with pharmacokinetic parameters listed in Table 1.

Total blood clearance (CLb) was estimated to be 132, 53, and 19 mL/min/kg in mice, rat, and Cynomolgus monkey, respectively (blood-to-plasma ratio of 0.6 across species). These values represented respectively ≥100, 69 and 41 % of hepatic blood flow in those species (Davies and Morris, 1993; Evans et al., 2006). After iv administration of CT7758 at 3 mg/kg to rodents and Cynomolgus monkey, plasma levels tended to decrease in a multiexponential manner with a terminal half-life of 1 h. In those species, plasma levels after oral administration at 30 mg/kg were extremely low with peak concentrations ≤ 0.15 µg/mL and oral bioavailability (F) ≤4%.

When compared to the above mentioned species, dog showed a quite different pharmacokinetic profile with lower blood clearance (1.78 mL/min/kg, i.e. 6 % of hepatic blood flow) and longer terminal t1/2 (2.4 h). Dog also showed higher levels after oral administration (Cmax up to 109 µg/mL) although quite variable between individuals (F values ranging from 7 to 55 %). Of notice, the volume of distribution in dog (i.e. Vz of 0.24 L/kg) was in the range of extracellular fluid volume, being much lower than in rodents or cynomolgus monkeys (1-5 L/kg).

The pharmacokinetic study in rat also incorporated portal vein sampling after oral dosing. The aim was to elucidate the low oral bioavailability observed in most species (e.g. 2 % in rat). By using the equation, Fa = [Qp*(AUCp – AUCs)]/dose where Qp is portal vein blood flow and AUCp and AUCs are the area under the curve determined in portal vein and systemic
circulation, respectively (Ward et al., 2001), the fraction of CT7758 absorbed (Fa) in rat was determined to be 3%. Comparison of portal vein and systemic levels predicted a moderate hepatic extraction ratio Eh of 0.55, from which a hepatic clearance CLH of 28 mL/min/kg was derived. This latter value is close to the observed total plasma clearance of 35 mL/min/kg. Overall, these data suggest that the low oral bioavailability in rat primarily results from restricted intestinal absorption, not from high hepatic first-pass. Also, data showed that total plasma clearance appears to be primarily hepatic in origin.

The excretion of [14C]-CT7758 was also measured in bile-duct cannulated male rat after single iv dosing at 3 mg/kg. A very low fraction of the radioactive dose (≤ 2.5 %) was recovered in urine, faeces, gastrointestinal tract and its content, ruling out renal excretion and intestinal secretion as elimination routes. This contrasts with the bile where 78% of the dose was recovered, mostly as unchanged CT7758 (collected over 6h period; n=4).

The allometric analysis illustrated in Fig 3 shows that mice, rat and cynomolgus monkey compared well with respect to total plasma CL, whereas dog appeared as an outlier. In an attempt to explain this discrepancy, species were compared for CT7758 plasma protein binding. Plasma protein binding was found to be 98-99 % irrespective of the species, which could not explain the observed interspecies variability in pharmacokinetic parameters (Table 3). On the other hand, correcting clearance values for bile flow rate normalized by per kg liver weight (Mahmood, 2005) provided more consistent allometric relationships across species (r² of 0.96, exponent of 0.58) and predicted a total plasma clearance in human of 0.41 mL/min/kg.

3.2. In vitro Pharmacokinetics of CT7758

The limited intestinal permeability of CT7758 was confirmed in vitro. The measured apical to basolateral flux in Caco-2 cells ranged from 0.68 to 1.34 x 10⁻⁶ cm/sec. Based on reference standards tested in similar conditions, CT7758 was predicted to be absorbed by 3 to 38 % in
human (Fig.4). As shown in Table 2, CT7758 showed polarized permeability suggesting active efflux. Also, the apical to basolateral permeability was demonstrated to increase as the pH in the apical compartment was decreased which was consistent with a lower ionization of CT7758 at more acidic pH.

In an attempt to explore the role of metabolism in the hepatic clearance, the transformation of $[^{14}C]$-CT7758 was measured by radio-HPLC following its incubation with rat and dog hepatocytes. The compound was demonstrated to be slowly metabolized, with an intrinsic metabolic clearance of as low as 4.5 and 0.65 µL/min/10^6 cells in rat and dog hepatocytes, respectively. When corrected for plasma protein binding, these in vitro values translated into predicted in vivo hepatic clearance of 0.27 and 0.023 mL/min/kg in rat and dog, respectively which remains considerably lower than the observed total plasma clearance in the same species.

Further in vitro assays were performed to investigate whether active uptake (as opposed to oxidative metabolism) could potentially account for the observed clearance of CT7758. Its uptake was measured in hepatocytes using the oil spin methodology. CT7758 was found to be rapidly taken up by mice and rat hepatocytes with an uptake CL_{int} of 2.0 and 5.3 ml/min/10^6 cells, respectively. Dog and human showed a lower uptake with 0.7 and 1.2 ml/min/10^6 cells, respectively (Table 3). Metabolic clearance of CT7758 was also assessed in hepatocytes suspension and demonstrated very low turn-over in rat hepatocytes (7 µL/min/10^6 cells) and no detectable turn-over (<1 µL/min/10^6 cells) in dog and human hepatocytes (Table 3). Once scaled-up taking into account plasma protein binding and blood-to-plasma ratio as described in materials and methods section, these in vitro uptake clearance values in animal species were found in good agreement with the total plasma clearance measured in vivo (Fig.5A, Table 3) which contrasted with the major under-prediction of the scaled clearance obtained from metabolic clearance. Human clearance was predicted to be 7.4 mL/min/kg, i.e. more
than 10-fold higher than the clearance predicted by allometry (after correction for bile flow rate). Additional \textit{in vitro} experiments were carried out to further investigate the mechanisms underlying the active uptake. First, as shown in Fig 5B, the uptake of CT7758 in rat hepatocytes was found to be a saturable high-affinity process with a K_m of 4.2 µM. In addition, MK571, a well known inhibitor of OATPs, was shown to inhibit the uptake of CT7758 in rat hepatocytes with an IC_{50} of 3.0 µM (Fig 5C). Finally, the uptake of 10 µM CT7758 in organic anion transporting polypeptide OATP1B1-expressed cells was found to be consistently higher than in the corresponding mock cells, with a response similar to the one reported for [^3H]-estrone-3-sulfate, a substrate for OATP1B1 (Nozawa et al., 2005) (Fig. 6A). Using the same engineered cells, CT7758 was demonstrated to inhibit the uptake of [^3H]-estrone-3-sulfate by 40 % at 1 µM and full blockade at 100 µM (Fig. 6B) further confirming an interaction with OATP1B1 transporter.

\subsection*{3.3. \textit{In vitro} Pharmacokinetics of CDP323}

CDP323, the ethyl ester prodrug of CT7758, was developed to circumvent potential intestinal absorption issues in humans. The \textit{in vitro} metabolism of [^14C]-CDP323 (5µM) was investigated in mice, rat, dog and human fresh hepatocytes. CDP323 was found to be rapidly cleared \textit{in vitro} with t_{1/2} of 21, 6, 58 and 16 min in mice, rat, dog and human hepatocytes, respectively (Fig. 7A). CT7758 formation accounted almost entirely for the rapid disappearance of CDP323 (Fig. 7B).

The \textit{in vitro} permeability of CDP323 was investigated in Caco-2 cells and compared to that of CT7758. CDP323 showed a higher flux than CT7758, both in the apical-to-basolateral and basolateral-to-apical directions (Fig. 8). CDP323 demonstrated non-linear apical-to-basolateral flux, complicating permeability value calculations. Also, these data should be interpreted with caution as CDP323 might have been partially hydrolyzed during the assay.
Indeed, hydrolytic enzymes can be released from Caco-2 brush border membranes, as reported elsewhere (Yuan et al., 2009; Brouwers et al., 2007; Chanteux et al., 2005). These in vitro data in the hepatocytes clearance assay showing efficient release of the active moiety combined with an improved mass transfer using the prodrug were encouraging and triggered additional in vivo pharmacokinetic studies.

3.4. In vivo Pharmacokinetics of CDP323

The pharmacokinetics of CDP323 has been evaluated following single oral administration to mice, rat, dog, and Cynomolgus monkey (Fig. 9 and Table 4). Plasma samples were examined for unchanged drug and CT7758.

After oral administration of CDP323, CT7758 was by far the most abundant circulating plasma component peaking between 0.5 and 1.5 h, irrespective of the species. These data suggested that CDP323 was rapidly absorbed and efficiently hydrolysed into CT7758. Plasma exposure of CT7758 showed a large species variability with dog $\approx$ rat $\approx$ mice $>$ cynomolgus.

In the tested dose range of 25-50 mg/kg, the estimated oral bioavailability (i.e. based on intravenous administration of CT7758 and assuming linear PK) was 29, 27, 8 and 0.3 % in mice, rat, dog and cynomolgus monkey, respectively. CDP323 increased the absorption of CT7758 by 5-10 fold in rodents, whereas no significant increase was observed in dog and monkey.
DISCUSSION

Poor intestinal absorption and extensive biliary clearance have been recurring issues in the development of small molecule VLA-4 antagonists (Davenport and Munday, 2007).

Several physicochemical properties have been discussed as potentially involved in the low intestinal absorption of VLA-4 antagonists, such as high molecular weight (Muller et al., 2001; Davenport and Munday, 2008), low lipophilicity degree (Chiba et al., 2007; Chiba et al., 2006a; Chiba et al., 2006b), presence of a carboxylate function (Davenport and Munday, 2007; Gong et al., 2008). Polar surface area (PSA, sum of hydrogen bond donor and acceptor groups) is another readily accessible descriptor used to predict intestinal absorption (Bergstrom et al., 2003; Veber et al., 2002) which could also play a role in VLA-4 antagonist pharmacokinetics (Davenport and Munday, 2007). The VLA-4 antagonist CT7758 discussed here is showing a PSA at 104, an oral bioavailability of 2% in rat and a fraction absorbed as low as 3%.

When compared to mice, rat and Cynomolgus monkey (F of 4, 2 and 0.2%, respectively), dog contrasted with a higher although variable oral bioavailability (23±27%). Such species difference is not uncommon for poorly absorbed drugs. A couple of polar compounds (e.g. acyclovir, nadolol, ranitidine, xamoterol) have been described as being better absorbed in dog than in the other species (Cheng et al., 2008; Chiou et al., 2000). As a rule, rat and monkey are reported as more reliable predictors of intestinal absorption in human (Chiou and Buehler, 2002; Chiou and Barve, 1998). Various mechanisms might account for higher absorption in dog, i.e. larger pore size and density and thus higher paracellular flux (He et al., 1998), different expression of efflux proteins (Bleasby et al., 2006), and differences in physiology and anatomy of GI tract (Cheng et al., 2008). One or several of these mechanisms may be responsible for the higher absorption of CT7758 observed in dogs as well as for the inter-individual variability observed in that species. Indeed, as expected for any carboxylic acid,
the membrane permeability of CT7758 was found to be pH-dependent. Akimoto (Akimoto et al., 2000) reported that the gastric pH of Beagle dogs was highly variable between individuals following administration of a placebo capsule. Variability was observed on the minimum gastric pH (range from 2.2 to 6.6) but also on the time to reach minimum that pH value after capsule administration (from 30 min to 1h30). This variability in gastric pH is likely to account for the observed variability in the oral absorption of CT7758 in dogs. Further investigations, beyond the scope of the present study, would be required to fully understand the mechanisms underlying the higher absorption and the highly variable pharmacokinetics of CT7758 in dogs.

Pharmacokinetic studies in Eisai hyperbilirubinaemic rats (i.e. lacking functional MRP2) and/or in rats coadministered with cyclosporine A (inhibitor of various transporters), as well as in vitro assays demonstrated that the rapid biliary clearance of VLA-4 antagonists in rat involved MRP2 (Cheng et al., 2008;Kamenecka et al., 2004;Tang et al., 2008;Tsuda-Tsukimoto et al., 2006). On the other hand, MRP2-mediated efflux into the bile alone cannot account for all the observed findings. Still, these compounds are rapidly cleared by biliary elimination. Together these findings imply active uptake transport into the liver, to compensate for their low membrane permeability. This hypothesis was previously confirmed for two VLA-4 antagonists demonstrated to act as substrate of hepatic organic anion transporting polypeptide. The present study similarly demonstrated that CT7758 is also a substrate of human OATP1B1.

As a confirmation, in vitro uptake assay of CT7758 in hepatocytes quantitatively predicted the in vivo total plasma clearance across species. The human plasma clearance predicted from the in vitro uptake assays fitted perfectly with the values subsequently measured in human volunteers after iv administration of CT7758 (5-7 versus 5 ml/min/kg, respectively, data on UCB files). This finding demonstrates a key role of active uptake in the pharmacokinetics of
VLA-4 antagonists and further highlights the value of *in vitro* uptake assays to support drug optimization and profiling (Chiba et al., 2009; Li et al., 2013).

Volume of distribution (Vz) of CT7758 was also significantly different between mouse (5.5 L/kg), rat (2.8 L/kg), dog (0.24 L/kg) and cynomolgus monkey (0.93 L/kg) which could not be explained by plasma protein binding difference which ranged between 97.6 and 99%. Healthy volunteers demonstrated low volume of distribution (Vz of 34L) of CT7758 following iv dosing of CT7758 at 10mg (data on UCB files). Overall, the observed human PK parameters of CT7758 (CL, Vz and T1/2 of 1.3h) suggested that cynomolgus monkey was more predictive than other species.

One might question whether the above mentioned species variability in CT7758 clearance and distribution might result from differences in the expression of organic anion transporter or differences in its substrate specificity. In humans there are two major liver specific OATP1B members, *i.e.* OATP1B1 and OATP1B3, which share 80% sequence homology and are both involved in the elimination of endogenous compounds as well as bulky protein-bound amphipathic organic drugs (Hagenbuch and Meier, 2004). Rodents and dog have each only one ortholog, oatp1b2 (DeGorter et al., 2009; Schwabedissen et al., 2007) and Oatp1b4, respectively (Gui and Hagenbuch, 2010). Although not yet fully explored, large species variability in organic anion transporting polypeptide-mediated drug uptake has been reported (Chu et al., 2013). Paclitaxel and digoxin are substrates of human OATP1B1/3 but are not transported by dog Oatp1b4 (Gui and Hagenbuch, 2010) nor by mice Oatp1b2 (Meyer Zu Schwabedissen et al., 2009; Smith et al., 2007). Conversely, $^3$H-taurocholate and $^3$H-estradiol 17β-glucuronide are much better transported by murine Oatp1b2 than by the human counterpart (Meyer Zu Schwabedissen et al., 2009). Pravastatin is a well recognized substrate of rodent Oatp1b2 (Meyer Zu Schwabedissen et al., 2009; Tokui et al., 1999), not of canine Oatp1b4 (Wilby et al., 2011). A recent study compared the uptake of pravastatin in
hepatocytes from various species (Li et al., 2013). Large differences were observed with the uptake clearance values ranked as follows: mouse > rat > monkey > human > dog (13, 5, 4, 2 and 1 µL/min/10^6 cells, respectively). Another study investigated the uptake of prototypical OATP substrates in rat and human hepatocytes (Menochet et al., 2012). The active uptake was on average 7-fold higher in rat than in human cells, with up to 21-fold difference for some compounds like rosuvastatin. On the other hand, dog Oatp1b4- and human OATP1B3-expressed cells showed broadly similar uptake clearance values against a set of eleven substrates (Wilby et al., 2011). Overall, these observations suggest that the liver uptake of OATP substrates in human is species dependent and that it could be predicted from different in vitro assays.

Other VLA-4 antagonists have been previously reported to show interspecies variability in their pharmacokinetics. A couple of antagonists share the same profile than CT7758 with much lower total plasma clearance and lower volume of distribution in dog than in rodent. The most striking examples include compound 14e (trans-4-[1-[[2,5-dichloro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetyl]-(4S)-methoxy-(2S)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid) (Muro et al., 2009) with a CL of 19.3, 1.7, 1.8 mL/min/kg in rat, dog and monkey, respectively. The volume of distribution follows the same ranking with 1.22, 0.16 and 0.22 L/kg, respectively. Another example is D01-4582 (4-[1-[3-chloro-4-[N’-(2-methylphenyl)-ureido]phenylacetyl]-(4S)-fluoro-(2S)-pyrrolidine-2-yl]-methoxybenzoic Acid) (Ito et al., 2009;Ito et al., 2007). Its CL was reported to be 31.5 and 3.7 mL/min/kg in SD rats and Beagle dogs, respectively, with Vdss of 0.47 and 0.14 L/kg.

In an effort to address the potential low intestinal absorption of CT7758, the ester prodrug CDP323 was developed. Many carboxylate-containing VLA-antagonists have been optimized as prodrugs. While in some cases prodrugs offered better intestinal permeability and increased exposure after oral (Huryn et al., 2004a;Huryn et al., 2004b;Reger et al., 2010;Venkatraman et
al., 2009; Dyatkin et al., 2005), some other attempts remained unfruitful or of limited benefit (Chiba et al., 2006a; Chiba et al., 2006b; Gong et al., 2008; Gong et al., 2006). As demonstrated with other ester prodrugs (Chanteux et al., 2003), the present data showed that CDP323 prodrug increased mass transfer across biological membranes when compared to the carboxylic acid derivative, CT7758. In addition, CDP323 also efficiently released \textit{in vitro} (Chanteux et al., 2014) and \textit{in vivo} its active moiety, CT7758, as already described earlier. However, the improvement of CT7758 absorption through the use of an ester prodrug, CDP323, was only observed in rodents while no or subtle increase were present in dog and monkey which could raise the question about the clinical relevance of such finding. However, the poor exposure observed in rodents when they were administered CT7758, was compromising the feasibility of toxicity studies in rodents and therefore the whole development program. The ability of this ester prodrug to significantly improved the exposure achieved in rodents was another key driver in the decision to progress this ester prodrug in clinical development even though some uncertainties remained about its potential to enhance the absorption and exposure of CT7758 in humans. The prodrug progressed into clinical development and demonstrated an efficient release of CT7758 with plasma levels of prodrug barely detectable (Baker et al., 2006) as expected from its high in vitro intrinsic clearance and showed a bioavailability of 14\% (compared to iv dosing of CT7758) (Nicolas, 2011). Afterwards, CDP323 entered a phase II clinical trial in subjects with relapsing forms of multiple sclerosis over 24 weeks. However, its development was discontinued in 2009 based on inadequate interim efficacy data in the Phase II study (Wolf et al., 2013).

In conclusion, CT7758 showed large species differences in its pharmacokinetic parameters. Many evidences point out the involvement of active uptake and efflux transporters as the main reason of these observed species differences in CT7758 disposition. Specifically, OATPs were shown to be involved in the transporter-mediated uptake in hepatocytes. \textit{In vitro}
uptake assays were proven to be helpful and useful to compare species differences and to predict the clinical situation. Since CT7758 demonstrated low intestinal permeability due to active efflux, the ester prodrug strategy was investigated. CDP323, an ethyl ester prodrug, showed some improvements in increasing mass transfer and was considered as a viable candidate for clinical development.
ACKNOWLEDGMENTS

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Chanteux, Prakash, Nicolas

Conducted experiments: Mancel, Gerin, Boucaut

Contributed new reagents or analytic tools: Staelens, Mancel

Performed data analysis: Chanteux, Staelens, Mancel, Gerin, Prakash, Nicolas

Wrote or contributed to the writing of the manuscript: Chanteux, Staelens, Mancel, Gerin, Nicolas
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Ito T, Takahashi M, Sudo K and Sugiyama Y (2009) Interindividual pharmacokinetics variability of the alpha(4)beta(1) integrin antagonist, 4-[1-[3-chloro-4-[N-(2-


LEGEND FOR FIGURES

**Fig 1.** Chemical structure of CT7758 and its ethyl ester prodrug, CDP323, with the position of $^{14}$C atom represented by a star (*).

**Fig.2.** Plasma concentrations versus time profile of CT7758 in male mice (solid circle), rats (open square), Beagle dogs (solid square) and Cynomolgus monkeys (open circle) after single iv dosing at 3 mg/kg (Panel A) or single oral dosing at 30 mg/kg (Panel B). Data expressed as mean ± SD of three animals.

**Fig.3.** Allometric scaling of total plasma CL of CT7758 across animal species (M: mice, R: rat, C: cynomolgus monkey, D: dog). Human prediction (H) is illustrated by the open symbol. Panel A: simple allometry. Panel B: plasma clearance values corrected for bile flow rate. Bodyweight of 20g, 250g, 3kg, 10 kg and 70 Kg were used for mice, rats, monkeys, dogs, and human, respectively.

**Fig.4.** Apical to basolateral permeability of CT7758 in Caco-2 cell monolayers. CT7758 (20 µM) and reference standards were measured for their *in vitro* permeability (apical to basolateral) in Caco-2 cells. CT7758 is represented using (◊) whereas (+) represented all the reference standards used to derive the fitted curve.

**Fig.5.** Panel A: Observed total plasma clearance of CT7758 after single iv dosing (solid bars; see Table 1 for details) compared with the hepatocyte uptake clearance scaled-up to the *in vivo* situation (open bars). Panel B: *in vitro* uptake of $^{14}$C]-CT7758 in fresh rat hepatocytes using the silicon oil centrifugation technique. Rat hepatocytes were incubated for 30s at increasing concentration of CT7758 and the amount of intracellular $^{14}$C]-CT7758 was determined according to method described in materials and methods section. Panel C: Inhibition of $^{14}$C]-CT7758 (5 µM) uptake into rat hepatocytes media by increasing concentration of MK571.
**Fig.6.** Panel A: Total CT7758 uptake in OATP1B1-transfected cells (solid symbols) when compared to mock cells (open symbols). Cells (OATP1B1-transfected and mock cells) were incubated with CT7758 at 5 µM. At appropriate incubation time, cells were washed and monolayers were collected in uptake medium and lysed for the determination of intracellular CT7758. Panel B: Inhibitory effect of CT7758 (solid bars) on the uptake of [³H]-estrone-3-sulfate in OATP1B1-transfected cells. Rifampicin 100 µM (open bar) was used as positive control.

**Fig.7.** Parent drug consumption (Panel A) and CT7758 formation (panel B) following incubation of [¹⁴C]-CDP323 (5 µM) with primary cultures of hepatocytes from rat (open circle), human (solid circle), mice (open triangle) and dog (solid triangle). Dotted lines correspond to monoexponential fitting used to compute *in vitro* t₁/₂ values. Human data are reported as mean ± sd of three donors.

**Fig.8.** [¹⁴C]-CDP323 (closed circle) and [¹⁴C]-CT7758 (open triangle) transport through Caco-2 monolayers. Panel A: Apical to Basolateral (A>B) transport Apparition of total radioactivity in receiver compartment when 10 µM [¹⁴C]-CDP323 or [¹⁴C]-CT7758 was added in donor compartment (compartment A). Panel B: Basolateral to Apical (B>A) transport. Apparition of total radioactivity in receiver compartment (compartment A) when 10 µM [¹⁴C]-CDP323 or [¹⁴C]-CT7758 was added in donor compartment (compartment B). Mean ± SD of three replicates are shown.

**Fig.9.** Plasma concentrations versus time profile of CT7758 after single oral dosing with CDP323 to mouse (solid circle; 50 mg/kg; 3 male and 3 females combined), rat (open square; 25 mg/kg; 6 males), Beagle dog (solid square; 50 mg/kg; 3 males and 3 females combined) and Cynomolgus (open circle; 50 mg/kg; 2 males). Data expressed as mean ± SD.
TABLES

TABLE 1

Mean pharmacokinetic parameters of CT7758 after single iv or oral dosing of CT7758
(n=3/sampling time)

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>C(_{\text{max}}) (µg/mL)</th>
<th>AUC(_{0-\infty}) (µg.h/ml)</th>
<th>T(_{1/2}) (h)</th>
<th>CL (mL/min/kg)</th>
<th>V(_z) (L/kg)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>3</td>
<td>iv</td>
<td>-</td>
<td>0.629</td>
<td>0.8</td>
<td>79</td>
<td>5.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>oral</td>
<td>0.139</td>
<td>0.206</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.3</td>
</tr>
<tr>
<td>Rat</td>
<td>3</td>
<td>iv</td>
<td>-</td>
<td>1.22</td>
<td>0.9</td>
<td>35</td>
<td>2.8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>oral</td>
<td>0.061</td>
<td>0.239</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Dog</td>
<td>3</td>
<td>iv</td>
<td>-</td>
<td>47.9</td>
<td>2.4</td>
<td>1.2</td>
<td>0.24</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>oral*</td>
<td>9.7/15.8/109</td>
<td>54.7/34.4/164</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7/8/55</td>
</tr>
<tr>
<td>Monkey</td>
<td>3</td>
<td>iv</td>
<td>-</td>
<td>3.95</td>
<td>1.0</td>
<td>11</td>
<td>0.93</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>oral</td>
<td>0.028</td>
<td>0.075</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* individual values reported since huge inter-individual variability
TABLE 2

*In vitro* permeability of CT7758 in Caco-2 cell monolayers. CT7758 was added to the donor compartment at the indicated concentration. The pH of the apical compartment was adjusted as indicated in the Table while the pH of the basolateral compartment was set at 7.4. Results are represented as mean ± SD (n=3)

<table>
<thead>
<tr>
<th>CT7758 (µM)</th>
<th>Apical pH</th>
<th>Apparent Permeability (nm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apical to Basolateral</td>
<td>Basolateral to Apical</td>
</tr>
<tr>
<td>20</td>
<td>5.0</td>
<td>26.8 ± 2.0</td>
</tr>
<tr>
<td>20</td>
<td>6.0</td>
<td>19.0 ± 1.5</td>
</tr>
<tr>
<td>20</td>
<td>6.5</td>
<td>13.4 ± 1.3</td>
</tr>
<tr>
<td>20</td>
<td>7.4</td>
<td>15.1#</td>
</tr>
<tr>
<td>1</td>
<td>6.5</td>
<td>7.1 ± 0.7</td>
</tr>
<tr>
<td>20</td>
<td>6.5</td>
<td>6.8 ± 1.7</td>
</tr>
<tr>
<td>100</td>
<td>6.5</td>
<td>9.4 ± 2.3</td>
</tr>
</tbody>
</table>

#n=2
### TABLE 3

In vitro metabolic and uptake clearance in mouse, rat, dog and human hepatocytes and their scaled hepatic clearance according to materials and methods section

<table>
<thead>
<tr>
<th>Species</th>
<th>In vitro clearance</th>
<th>CLint (µL/min/10⁶ hep)</th>
<th>fu</th>
<th>B/P</th>
<th>Scaled Hepatic Clearance (mL/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Metabolic</td>
<td>NP</td>
<td>0.024</td>
<td>0.8</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>uptake</td>
<td>2000</td>
<td>0.024</td>
<td>0.8</td>
<td>62</td>
</tr>
<tr>
<td>Rat</td>
<td>Metabolic</td>
<td>7.2</td>
<td>0.01</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>uptake</td>
<td>5274</td>
<td>0.01</td>
<td>0.6</td>
<td>40</td>
</tr>
<tr>
<td>Dog</td>
<td>Metabolic</td>
<td>&lt;1</td>
<td>0.01</td>
<td>0.44</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>uptake</td>
<td>702</td>
<td>0.01</td>
<td>0.44</td>
<td>8.3</td>
</tr>
<tr>
<td>Human</td>
<td>Metabolic</td>
<td>&lt;1</td>
<td>0.01</td>
<td>0.6</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>uptake</td>
<td>1160</td>
<td>0.01</td>
<td>0.6</td>
<td>7.4</td>
</tr>
</tbody>
</table>

NP : Not Performed
TABLE 4

Mean pharmacokinetic parameters of CDP323 and CT7758 after single oral dosing of CDP323 (n=3/sampling time) given as a suspension

<table>
<thead>
<tr>
<th>Species</th>
<th>Oral Dose (mg/kg)</th>
<th>Pharmacokinetic Parameters of CDP323</th>
<th>Pharmacokinetic Parameters of CT7758</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cmax (µg/mL)</td>
<td>AUC_{0-∞} (µg.h/mL)</td>
</tr>
<tr>
<td>mouse^a</td>
<td>50</td>
<td>1.28</td>
<td>3.07</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>3.24</td>
<td>8.29</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>11.1</td>
<td>59.9</td>
</tr>
<tr>
<td>Rat</td>
<td>25</td>
<td>1.31</td>
<td>2.78</td>
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<tr>
<td>Dog^a</td>
<td>10</td>
<td>4.8</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>14.6</td>
<td>64.4</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>20.5</td>
<td>162</td>
</tr>
<tr>
<td>Monkey</td>
<td>50</td>
<td>0.0447</td>
<td>0.228</td>
</tr>
</tbody>
</table>

^a mean of three males and 3 females combined

^b Oral bioavailability values are based on CT7758 only and used exposure data after 3 mg/kg CT7758 iv as detailed in Table 1 (*i.e.* assumed linear PK after iv).

nd : not detected
Figure 1

CT7758
MW: 521.42
Log D (pH 7.4) : 0.74

CDP323
MW: 549.47
Log D (pH7.4) : 5.6
Figure 3

(A) Total plasma clearance Cl (mL/min) vs. Bodyweight (kg)

(B) Total plasma clearance Cl (mL/min) vs. Bodyweight (kg)
Figure 6

(A) CT7758 uptake (µM in cell lysate) over time (min) for OATP1B1 cells (closed circles) and mock cells (open circles).

(B) [% control] of [3H]-estrone-3-sulfate uptake at different concentrations (1 µM, 10 µM, 100 µM).
Figure 9

CT7758 (ng/mL) vs. Time (h)

Data points represent the mean ± SEM of at least three independent experiments.