In Vitro Investigation, Pharmacokinetics, and Disposition of Imeglimin, a Novel Oral Antidiabetic Drug, in Preclinical Species and Humans^S

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ABSTRACT

Imeglimin is a novel oral antidiabetic drug for treatment of type 2 diabetes that targets mitochondrial bioenergetics. Its pharmacokinetics absorption characteristics, metabolism, distribution, and elimination were assessed through several in vitro and in vivo experiments in both animals and humans. Its potential to induce drug-drug interactions was also extensively assessed. Imeglimin is a small cationic compound with an intermediate intestinal permeability. Its absorption mechanism involves an active transport process in addition to passive paracellular absorption. Absorption was good (50%–80%) in vivo across several species but decreased with increasing dose, probably because of saturation of active transport. After absorption, imeglimin was rapidly and largely distributed to internal organs. Plasma protein binding was low, which can explain the rapid distribution to organs observed in all species. In animals and humans, imeglimin was largely excreted unchanged in urine, indicating a low extent of metabolism. Unchanged drug was the main circulating entity in plasma, and none of the identified metabolites were unique to human. Imeglimin renal clearance was higher than creatinine clearance, indicating that it was actively

secreted into urine. There was no evidence that it had the potential to cause cytochrome P450 inhibition or induction. It was shown to be a substrate of organic cation transporter (OCT) 1, OCT2, multidrug and toxin extrusion (MATE) 1, and MATE2-K and an inhibitor of OCT1, OCT2, and MATE1; as a consequence, corresponding clinical drug-drug interaction studies were performed and confirmed the absence of relevant interactions with substrates or inhibitors of these transporters.

SIGNIFICANCE STATEMENT

Imeglimin is absorbed through a passive and active mechanism, which can be saturated. It is rapidly and largely distributed to internal organs and mainly excreted unchanged in urine. It is poorly metabolized and has no cytochrome P450 inhibition or induction potential. Imeglimin is a substrate of MATE2-K and also a substrate and an inhibitor of OCT1, OCT2, and MATE1 transporters; however, there are no clinically significant interactions when imeglimin is coadministered with either a substrate or an inhibitor of these transporters.

Introduction

Type 2 diabetes mellitus (T2DM) is a widespread disease, with an increase in prevalence linked to obesity and sedentary lifestyle. The International Diabetes Federation has provided a global estimation of diabetes prevalence for 2017: 451 million people suffer from diabetes, and this prevalence is expected to increase in the future (Cho et al., 2018). This supports the need for well tolerated and potent drugs to enable long-term treatment and modify disease progression. Imeglimin is a novel oral antidiabetic drug targeting T2DM treatment that differs from other marketed drugs via

its novel structure and new mechanism of action (Johansson et al., 2020).

Imeglimin is the first in a new class of tetrahydrotriazine molecules called “glimins”; it targets mitochondrial bioenergetics, allowing for improvement in β -cell function and insulin secretion in response to glucose (Pirags et al., 2012; Pacini et al., 2015). It improves mitochondrial function by modulating mitochondrial respiratory chain complex activities while decreasing reactive oxygen species production and delaying the mitochondrial permeability transition pore’s opening in oxidative stress conditions.

Several clinical trials were performed in Caucasian and Japanese patients with T2DM; these demonstrated that imeglimin is efficacious as a monotherapy or add-on therapy, with an adequate safety and tolerability profile (Pirags et al., 2012; Fouqueray et al., 2013, 2014, 2015; Dubourg et al., 2017). These studies also showed that the clinical

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ABBREVIATIONS: A-B, apical-to-basal; ADME, administration, distribution, metabolism, and excretion; AUC, area under the plasma concentration-time curve; AUC_{0–last}, area under the plasma concentration-time curve from time zero to last measurable concentration; AUC_{0–∞}, area under the plasma concentration-time curve from time zero to infinity; B-A, basal-to-apical; BCRP, breast cancer resistance protein; CL, clearance of drug from plasma; CL_R, renal clearance; DDI, drug-drug interaction; E3S, estrone-3-sulfate; HEK, human embryonic kidney; hOCT, human organic cation transporter; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LLC-PK1, Lilly Laboratories cell-porcine kidney 1; LSC, liquid scintillation counting; MATE, multidrug and toxin extrusion transporter; MPP+, *N*-methyl-4-phenylpyridinium; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; P-gp, P-glycoprotein; PK, pharmacokinetics; *t*_{1/2}, terminal elimination half-life; T2DM, type 2 diabetes mellitus; *t*_{max}, time to reach maximal concentration; V_{ss}, volume of distribution; V_{ss}/F, apparent volume of distribution.

Fig. 1. Structural formula of imeglimin salt. *Position of ^{14}C radiolabel.

therapeutic dose for imeglimin ranged between 1000 mg twice a day and 1500 mg twice a day in Japanese and Caucasian patients with T2DM.

As with any new chemical entity, it is important to identify and quantify all relevant metabolites and elimination pathways for assessment of possible metabolite pharmacology, safety (Gao *et al.*, 2013, Center for Drug Evaluation and Research (CDER, 2020; ICH (2009))), clearance mechanisms, and potential drug-drug interactions (DDIs) (Committee for Human Medicinal Products (CHMP, 2012, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), 2020, CDER, 2020b)). Thus, we report here 1) the assessment of absorption, distribution, metabolism, and excretion (ADME) profiles of [^{14}C]imeglimin in animals and humans, 2) the identification of enzymes and transporters involved in its pharmacokinetics, and 3) the assessment of the potential risk of DDI with imeglimin as a perpetrator drug.

Methods

Chemical and Equipment

For preclinical studies, imeglimin radiolabeled test substance (chemical purity >98%) was synthesized at Merck KGaA, Grafting, and Pharmaron UK Ltd. (Cardiff). Metabolite reference compounds EMD 90076, EMD 27355, EMD 601811, EMD 602796, and EMD 647302 were provided by the medicinal chemistry group of Merck KGaA, Darmstadt, Germany, or medicinal chemistry at Merck KGaA, France. Imeglimin drug substance was manufactured by Merck KGaA or Poxel for the different experiments detailed below. The chemical structure of the compound and the radiolabel are shown in Fig. 1.

In Vitro Investigations

In Vitro Permeability Studies. The Caco-2 cell line (TC7-clone) was a gift of Prof. Dr. A. Zweibaum (INSERM, U-178 Villejuif, France). Cell suspensions in Dulbecco's modified Eagle's medium with Gluta-max, 10% FBS, 1% non essential amino acids, and 1% Penicillin/Streptomycin/FBS/DMSO (v/v/v = 7:2:1) were cryopreserved and stored in liquid nitrogen in batches of approximately 2×10^6 cells. Caco-2 cells used during this study were from total passage numbers 20–34. Caco-2 cell monolayers (TC7-clone) were prepared by plating 10^5 cells/cm 2 in transwell plates and incubating in a CO $_2$ -containing, water-equilibrated atmosphere at 37°C for 7 to 8 days (21 days for the paracellular permeation experiments). Tightness of monolayers was confirmed by transepithelial electrical resistance measurements in each well before and after the experiment. For investigation of its paracellular permeation, [^{14}C]imeglimin was added to the apical compartment at various concentrations (3–300 μM) with and without EGTA (2 mM). In addition, [^3H]cimetidine, a drug known to be subject to paracellular

permeation, was included as a positive control in these experiments (Zhou *et al.*, 1999). Contribution of paracellular permeation was calculated according to the following equation:

$$F_{\text{paracell.}} = (\text{Papp}^{+\text{EGTA}} - \text{Papp}^{-\text{EGTA}}) / \text{Papp}^{+\text{EGTA}} \times 100,$$

where Papp is the apparent permeability and $F_{\text{paracell.}}(\%)$ is the contribution of the paracellular permeation to the overall permeation under the in vitro conditions of the model.

A study was also performed to investigate whether imeglimin is a substrate of P-glycoprotein (P-gp). P-gp has been proven to be involved in various DDIs in humans (Ambudkar *et al.*, 1999). To evaluate whether imeglimin is a substrate of P-gp in vitro, [^{14}C] imeglimin was added either to basal or to apical compartments at various concentrations (1.0–1000 μM), and its time-dependent appearance in the other compartment was monitored by measuring aliquots with liquid scintillation counting (LSC). Integrity of the cell model and expression of P-gp was verified by transport experiments with digoxin, which was used as P-gp reference substrate. Testosterone was also used as a high-permeability reference substance. As imeglimin is a small cationic drug, transport through organic cation transporters (OCTs) was suspected. [^{14}C]Imeglimin (10 μM) was incubated with or without the OCT inhibitor decynium (2 μM).

To determine whether imeglimin is a substrate of breast cancer resistance protein (BCRP), the effect of the BCRP-specific inhibitor (30 μM novobiocin) was investigated on the bidirectional permeability of imeglimin at 3 mM.

In Vitro Metabolism in Human and Rat Subcellular Fractions.

The in vitro metabolism of imeglimin at 1, 10, and 100 μM was investigated in tissue homogenates and subcellular fractions of organs and tissues known to contain cytochrome P450 and other enzyme systems capable of metabolizing xenobiotics—namely, lung, liver, kidney, and intestine. For this purpose, incubations with both S9 mix and microsomes from liver, lung, intestine, and kidney derived from human and Wistar rat were carried out. The basic incubation mixtures contained [^{14}C]imeglimin (1, 10, and 100 μM) and 2 mM NADP, 3.3 mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphatedehydrogenase, 3.3 mM magnesium chloride, and 50 mM potassium phosphate buffer (pH 7.4), as well as either S9 (1 mg protein/ml) or microsomes (0.5 mg protein/ml). After a preincubation period (7 minutes at $37 \pm 2^\circ\text{C}$), the reactions were initiated by the addition of the appropriate application solution. The final individual incubation volume was 1 ml (200 μl per time point plus additional 200 μl). Incubations were performed for 0, 15, 30, and 60 minutes at $37 \pm 2^\circ\text{C}$. The reactions were then terminated by removal of a 200- μl aliquot and its addition to 0.1 ml ice-cold acetonitrile. The resulting protein precipitates in the stopped aliquots were removed in a centrifuge step at 3000 rpm. All incubations containing experimental components (including negative controls) were carried out in triplicate. The supernatants obtained after protein precipitation and centrifugation were stored frozen (at -20°C) prior to further analysis.

Binding to Plasma Protein and Blood Cell Partitioning. The extent of in vitro protein binding and plasma/blood cell partitioning of [^{14}C]imeglimin was determined in rat, dog, and human.

Plasma from all species was spiked with [^{14}C]imeglimin to achieve nominal concentrations of 1, 10, and 100 μM . Triple portions were subjected to equilibrium dialysis at $\sim 37^\circ\text{C}$ against phosphate-buffered saline (pH 7.4; Thermo Fisher Scientific, Japan) for 6 hours. After dialysis, duplicate aliquots from each cell half were analyzed by LSC to determine the concentration of radioactivity in each compartment and the percentage of protein binding. Quadruple blood samples from all species were spiked with [^{14}C]imeglimin at two concentrations (1 and

100 μM) and incubated at $\sim 37^\circ\text{C}$ on a rotary mixer for 4 hours. Aliquots were taken for determination of total radioactivity or transferred to hematocrit tubes for determination of the packed cell volume; the remaining blood was centrifuged at 2000g for 15 minutes, and duplicate aliquots of plasma were analyzed by LSC.

Determination of Substrate Potential with Hepatic or Renal Transporters. Several studies were performed to characterize the in vitro affinity of imeglimin to hepatic and renal transporters.

The transport of imeglimin through OCT1 was assessed by using human embryonic kidney (HEK) 293 cells expressing solute carrier transporters. Uptake was investigated to evaluate kinetic parameters including maximal transport velocity (V_{max}), apparent affinity (K_m), and intrinsic uptake clearance. Imeglimin was added to the cells at 10–3000 μM and transport was stopped after 2 minutes. Metformin was used as control substrate at 10 μM .

To assess whether imeglimin is an OCT2 substrate, transport assays were performed using HEK cells stably expressing human OCT2 (hOCT2) and vector-transfected control cells. To characterize activity in hOCT2-expressing HEK cells, *N*-methyl-4-phenylpyridinium (MPP+) was used as a control substrate at 1 μM . Uptake by hOCT2 was investigated to evaluate kinetic parameters: V_{max} , K_m , and intrinsic uptake clearance. Imeglimin was added to the cells at 10–1500 μM . The cells were incubated with ^{14}C - or ^3H -labeled compounds, and accumulation within cells was quantified by LSC.

Substrate assays of imeglimin with human MATE1, MATE2-K, organic anion transporter (OAT) 1, and OAT3 were then performed. The in vitro substrate properties of imeglimin with human MATE1, MATE2-K, OAT1, and OAT3 transporters were tested at two concentrations of imeglimin (0.1 and 1 mM) at two incubation time points (2 and 20 minutes). A 1 mM maximum tested dose was selected to cover a concentration range of 50-fold versus the unbound maximum plasma concentration (7.5 μM); considering a therapeutic dose of 1000 mg, this concentration must be at least 500 μM ($50 \times 7.5 = 375 \mu\text{M} \sim 500 \mu\text{M}$) (MHLW, 2018, (CDER, 2020b)). Metformin (10 μM), *p*-aminohippuric acid (5 μM), and estrone-3-sulfate (E3S at 1 μM) were used as substrates for MATE1 and MATE2-K, OAT1, and OAT3 experiments, respectively. Pyrimethamine (1 μM), benzbromarone (300 μM), and probenecid (500 μM) were used as reference inhibitors for MATE1 and MATE2-K, OAT1, and OAT3 experiments, respectively.

In Vitro Assessment of DDI Potential

Cytochrome P450 Inhibition Study. The ability of imeglimin to inhibit the major cytochrome P450 enzymes (namely, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) in vitro was evaluated with the aim of ascertaining the potential of this compound to inhibit the metabolism of concomitantly administered drugs. Inhibitory effects of imeglimin on cytochrome P450 activities were examined in human liver microsomes in the presence of NADPH.

The marker activities were as follows: CYP1A2, phenacetin *O*-deethylation; CYP2B6, bupropion hydroxylation; CYP2C8, paclitaxel 6 α -hydroxylation; CYP2C9, diclofenac 4'-hydroxylation; CYP2C19, (*S*)-mephenytoin 4'-hydroxylation; CYP2D6, bufuralol 1'-hydroxylation; and CYP3A4, midazolam 1'-hydroxylation and testosterone 6 β -hydroxylation. Positive control substances were as follows: CYP1A2, furafylline and α -naphthoflavone; CYP2B6, ticlopidine hydrochloride; CYP2C8, montelukast sodium and gemfibrozil 1-*O*- β -glucuronide; CYP2C9, sulfaphenazole and tienilic acid; CYP2C19, tranilcypromine hydrochloride and *S*-fluoxetine hydrochloride; CYP2D6, quinidine and paroxetine hydrochloride; and CYP3A4, ketoconazole and verapamil hydrochloride.

The tested dose must cover a concentration of 50-fold versus the unbound maximum plasma concentration (7.5 μM); considering a therapeutic dose of 1000 mg, this concentration must be 500 μM ($50 \times 7.5 = 375 \mu\text{M} \sim 500 \mu\text{M}$) (MHLW, 2018, (CDER, 2020b)). For CYP3A4, the tested dose must cover the maximum expected concentration in the intestinal lumen (0.1-fold the maximum dose on one occasion/250 ml). The maximum concentration must be 3000 μM ($0.1 \times 1500 \text{ mg}/250 \text{ ml} = 0.1 \times 31.3 \text{ mM} = 3.13 \text{ mM} \sim 3000 \mu\text{M}$) (MHLW, 2018, (CDER, 2020b)). To estimate the inhibition constant (K_i) from IC_{50} , a further 2-fold greater maximum concentration (1000 and 6000 μM) should be set, as the substrate concentration is close to its K_m . The concentrations tested ranged from 0.1 to 1000 μM for all cytochrome P450 enzymes, except for CYP3A4, for which imeglimin concentrations tested were higher (up to 6000 μM). After the incubation period, marker substrate was added at a concentration approximately equal to K_m (solubility permitting), and the residual enzyme activity was measured. In addition, time-dependent inhibitory effects of imeglimin on cytochrome P450 activities with 30-minute preincubation were examined. Known metabolism-dependent inhibitors of the cytochrome P450 enzymes investigated were included as positive controls.

Cytochrome P450 Induction Studies. Imeglimin was evaluated for its induction potential with several cytochrome P450 isoforms (CYP1A2, CYP2B6, and CYP3A4) by the mRNA expression levels of these cytochrome P450 isoforms in cryopreserved human hepatocytes from three individual donors after once-daily treatment with imeglimin at 0 (solvent control), 20, 60, and 120 μM for 48 hours. Induction potential was evaluated with the fold change in mRNA expression from solvent control and in comparison with positive control inducers. Positive control inducers 50 μM omeprazole, 2000 μM phenobarbital, and 25 μM rifampicin were used for CYP1A2, CYP2B6, and CYP3A4, respectively.

Transporter Inhibition Study. The in vitro inhibition potential of imeglimin with the human MATE1, MATE2-K, OAT1, OAT3, organic anion-transporting polypeptide (OATP) 1B1, and OATP1B3 transporters was tested at 0.1 and 1 mM concentrations of imeglimin. The tested concentration must cover the estimated maximum concentration at the inlet to the liver for hepatic transporters ($I_{\text{in,max}}$), which is calculated as follows: $I_{\text{in,max}} = [C_{\text{max}} + (F_a \times F_g \times k_a \times \text{Dose})/Q_h/R_B] \times f_{\text{up}}$, where F_a is the fraction absorbed, F_g is the intestinal bio-availability, k_a is the absorption rate constant, Q_h is the liver blood flow, R_B is the blood-to-plasma concentration ratio and f_{up} is the unbound fraction in plasma. Considering the maximum therapeutic dose of 1500 mg, the concentrations should encompass 15 μM [$(10 + (0.3 \times 0.1 \times 7826)/97/0.48) \times 0.936 \sim 15 \mu\text{M}$]. The tested concentrations must cover 10 or 50 times the maximum unbound plasma concentration for OAT1, OAT3, MATE1, and MATE-2K, respectively. Considering a therapeutic dose of 1500 mg, the concentrations should encompass 100 and 500 μM (MHLW, 2018, (CDER, 2020b)). Uptake experiments were performed using Madin-Darby canine kidney cells II or HEK293 cells stably expressing the respective uptake transporters. Cells were cultured at $37 \pm 1^\circ\text{C}$ in an atmosphere of 95:5 air/ CO_2 and were plated into standard 96-well tissue culture plates. Before the experiment, the medium was removed, and the cells were washed twice with 100 μl of assay buffer. In OAT3 experiments, cells were washed with 100 μl of HK buffer containing 5 mM glutaric acid (pH 7.4) and then were incubated at 37°C with HK buffer containing 5 mM glutaric acid (pH 7.4) for 20 minutes. Uptake experiments were carried out at $37 \pm 1^\circ\text{C}$ in 50 μl of assay buffer containing the probe substrate and imeglimin for 15 minutes (MATE1/MATE2-K), 2 minutes (OAT1), and 1 minute (OAT3). In the case of OATP1B1 and OATP1B3, cells were cultured at 37°C in a CO_2 incubator and plated into standard 24-well tissue culture

plates. Before the experiment, cells were washed with 500 μ l of 2-[4-(2-Hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid-Krebs-Henseleit buffer and then preincubated at 37°C for 30 minutes with preincubation medium containing imeglimin. Uptake experiments were carried out at 37°C in 250 μ l of assay buffer containing the probe substrate and imeglimin or solvent for 2 minutes.

A study was performed to evaluate the inhibitory effect of imeglimin after 2 hours of incubation at 1, 2, and 3 mM on P-gp in P-gp-expressing Lilly Laboratories cell-porcine kidney 1 (LLC-PK1) cells. An imeglimin concentration of 3 mM was used to cover the maximum expected concentration in the intestinal lumen (0.1-fold the maximum dose on one occasion/250 ml) (MHLW, 2018, (CDER, 2020b)). Remaining activity was calculated from the following equations:

$$\text{Remaining activity (\%)} = 100 \times \frac{(NFR_{TC} - 1)}{(NFR_{VC} - 1)},$$

with NFR_{TC} and NFR_{VC} corresponding to net flux ratio in the presence of the test substance and in the vehicle control, respectively.

$$NFR = \frac{\left(\frac{P_{app,B \rightarrow A}}{P_{app,A \rightarrow B}} \right) \text{ in transport expressing cells}}{\left(\frac{P_{app,B \rightarrow A}}{P_{app,A \rightarrow B}} \right) \text{ in Mock cells}}$$

Vesicular transport assays were performed with inside-out membrane vesicles prepared from cells overexpressing human ATP-binding cassette transporters. Imeglimin was incubated with E3S (1 μ M) as probe BCRP substrate for 1 minute. Ko134 (1 μ M) was used as reference inhibitor.

The inhibitory potency of imeglimin toward hOCT1-expressing HEK cells was determined using [3 H]MPP+ as a substrate. For IC_{50} studies, MPP+ was used at a concentration of 0.1 μ M because at this nonsaturating substrate concentration, the ratio of MPP+ concentration to the K_m of MPP+ (determined $K_m = 19.9 \mu$ M) was less than 1%, and K_i is virtually identical with IC_{50} . Cells were incubated with 0.1 μ M [3 H]MPP+ for 2 minutes at 37°C in the presence of varying concentrations of imeglimin (10–1000 μ M), and the accumulation in cells was quantified by LSC.

The inhibitory potency of imeglimin toward hOCT2-expressing HEK cells was determined using [3 H]MPP+ as a substrate. Cells were incubated with 0.1 μ M [3 H]MPP+ for 2 minutes at 37°C in the presence of varying concentrations of imeglimin (10–1000 μ M), and the accumulation in cells was quantified by LSC. The imeglimin tested concentrations cover 10-fold the maximum unbound plasma concentration (MHLW, 2018, (CDER, 2020b)).

Animal and Human 14 C ADME Studies

Study Designs. Dog and rat ADME studies were performed to assess the absorption, pharmacokinetics (PK), and routes and rates of excretion of [14 C]imeglimin after oral and intravenous administration (5 mg/kg).

Additionally, based on a high-dose oral administration (100 mg/kg) to both rats and dogs, the identity of metabolites, the relative proportions of [14 C]imeglimin, and its radiolabeled metabolites in plasma (only plasma for rats), urine, and feces were determined.

All studies were conducted in conformity with the guidelines. Male Wistar rats (HsdBrHan:WIST and Hsd/Cpb:WU, 201–288 g) and a Lister-Hooded (CrI:Lis BR, 197–220 g) strain were obtained from Harlan (UK) and Charles River (UK), respectively. Female beagle dogs (7.7–8.7 kg) were obtained from Harlan (France). Dogs were fasted overnight before dosing and fed again 4 hours after administration, whereas rats were not fasted. All animals had free access to water.

Rats received a single intravenous dose (5 mg/kg) as a bolus injection via a lateral tail vein or a single oral dose (5 or 100 mg/kg) by gavage of [14 C]imeglimin. For excretion balance at 5 mg/kg, the radioactive dose was in the range 6.29–6.69 MBq/kg. For pharmacokinetic and metabolite profiling, the radioactive doses used were between 1.06 MBq/kg (low dose) and 4.45 MBq/kg (high dose).

Dogs received one intravenous dose (5 mg/kg), administered via a cephalic vein, and one single oral dose (5 mg/kg) in a crossover design or a single higher oral dose (100 mg/kg) of [14 C]imeglimin. Radioactive doses were 0.5 MBq/kg (low doses) and 1 MBq/kg (high dose).

For rats and dogs, oral doses were administered by gavage. For intravenous and oral administrations, the substance was dissolved in 0.9% NaCl solution.

The clinical study was a phase I, single-center, open-label, single-dose ADME study in healthy male subjects. Six subjects (aged between 30 and 60 years, inclusive, body mass index 18.5–29.9 kg/m²) received a single oral dose of 1000 mg [14 C]imeglimin (containing 3.7 MBq 14 C radioactivity) as an oral solution after a 10-hour overnight fast. This study was approved by an independent ethics committee (Stichting Beoordeling Ethiek Bio-Medisch Onderzoek, Assen, The Netherlands) and by the competent authority (Central Committee on Research Involving Human Subjects or, in Dutch, Centrale Commissie Mensgebonden Onderzoek) in accordance with Dutch regulations. The study was conducted in accordance with the principles of the Declaration of Helsinki and in compliance with the International Conference on Harmonization E6 Guideline for Good Clinical Practice (Committee for Proprietary Medicinal Products guideline CPMP/ICH/135/95), and compliant with the European Clinical Trial Directive: Directive 2001/20/EC. All subjects signed the informed consent form during the prescreening visit.

Sampling. For rats, blood samples were obtained at the following time points postdose: predose, 0.1 and 0.25 (only for intravenous administration), 0.5, 1, 2, 4 and 6 (except at high oral dose), 8, 12 (except at high oral dose), 24 and 48 (except at high oral dose) hours.

At each sampling time, 0.5 ml of blood was withdrawn from the sublingual vein and transferred into heparinized tubes. The samples were centrifuged for 3 minutes at about 12,000g.

In total, 0.1 ml of the supernatant plasma was mixed with 10 ml Omni-Szintisol for determination of total radioactivity; 0.3 ml of the supernatant plasma from the high-dose animals was stored at –20°C for metabolism, and 0.3 ml of the supernatant plasma was stored at –20°C for imeglimin concentration determination. For metabolism profiling, pooled samples were prepared from four animals at the following time points: predose, 0.5, 1, 2, 8, and 24 hours after dosing. Further details for sampling are given in the Supplemental Data.

For dogs, blood samples were obtained at the following time points postdose: predose, 0.1 (only for intravenous administration), 0.25, 0.5 (except at high oral dose), 1, 2, 4 (except at high oral dose), 6, 8 (except at high oral dose), 24 and 48 (except at high oral dose) hours. Blood samples (5 to 6 ml) were withdrawn from the jugular vein into heparinized tubes and centrifuged (2500g for 10 minutes at ~+5°C). Plasma specimens were aliquoted as follows: 0.5 ml for total radioactivity determination, 1 ml for imeglimin determination (only for low doses), 1 ml for metabolic profiling, and 1.5–2 ml for metabolite identification (only for high dose). Samples were stored at –20°C and at –80°C for metabolic profiling and metabolite identification samples. For metabolite profiling, pooled samples of plasma were prepared from three animals at the following time points: 0.25, 1, 2, and 6 hours after dosing. Urine samples were pooled by collection period: 0–8, 8–24, 24–48, and 24–48 hours. Feces samples were pooled by collection period: 0–8, 8–24, and 24–48 hours. Further details for sampling are given in the Supplemental Data. For

TABLE 1
Apparent permeability of [^{14}C]imeglimin and reference substances in Caco-2 cell monolayers (TC7-clone)

Substrate	Concentration (μM)	$P_{\text{app,A-B}}$ ($10^{-6}\text{ cm}^2\text{ s}^{-1}$)	$P_{\text{app,B-A}}$ ($10^{-6}\text{ cm}^2\text{ s}^{-1}$)	$P_{\text{app,B-A}}/P_{\text{app,A-B}}$
Imeglimin	1	3.81	0.500	0.131
	3	5.09	0.910	0.179
	10	3.72	0.536	0.144
	30	3.41	0.752	0.221
	100	1.57	0.461	0.294
	300	1.06	0.547	0.514
	1000	0.486	0.397	0.817
Digoxin	5	0.669	12.7	19.4
Testosterone	10	15.0	17.6	1.18
Imeglimin + decynium	10	2.55	0.714	0.28

P_{app} , apparent permeability.

metabolite identification, pooled samples of plasma were prepared from three animals at the following time points: 0.25, 1, 2, and 6 hours after dosing; urine, the collection periods were 0–8 and 24–48 hours; for feces, collection periods of 24–48 hours were employed. Further details for sampling are given in the Supplemental Data.

For the excretion balance in dogs and rats, urine and feces were collected into preweighed containers over the following time periods after dose administration: predose, 0–8, 8–24, 24–48, 48–72, 72–96, and 96–120 hours postdose. The total weight of the collected urine and feces was recorded.

In the clinical study, the blood sampling (12 ml) for analysis of ^{14}C radioactivity and imeglimin was scheduled at the following time points and collected in heparin-containing tubes: predose, 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120, and 144 hours postdose. For the analysis of ^{14}C radioactivity and imeglimin in plasma, samples were centrifuged within 30 minutes at 4°C at 1500g for 10 minutes and stored at $-20^\circ\text{C} \pm 5^\circ\text{C}$ until analysis. For the analysis of ^{14}C radioactivity in blood, samples were aliquoted and stored at -20°C until analysis.

For blood-to-plasma distribution, samples of 2 ml of blood for the assessment of hematocrit were taken using potassium EDTA as the anticoagulant. Blood was collected on day 1 predose and 1, 3, and 6 hours postdose.

For metabolite pattern assessment in human plasma, 7-ml blood samples were collected in sodium heparin tubes, and the samples were immediately cooled at 4°C and centrifuged within 30 minutes at 4°C at 1500g for 10 minutes. A minimum of 3 ml of plasma was rapidly transferred to a polypropylene tube and stored immediately at $-20^\circ\text{C} \pm 5^\circ\text{C}$ until analysis. Blood sampling for metabolites was scheduled at the following investigation times: predose, 1, 3, 6, 8, 12, 24, 48, hours postdose. Further details for sampling are given in the Supplemental Data.

For the analysis of ^{14}C radioactivity and metabolite patterns, urine samples were collected. After drug administration, urine was collected from all subjects according to the following time intervals: 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–72, 72–96, 96–120, and 120–144 hours postdose. Further details for sampling are given in the Supplemental Data.

After drug administration, feces were collected from all subjects according to the following time intervals: 0–24, 24–48, 48–72, 72–96, 96–120, and 120–144 hours postdose. All feces samples were homogenized in 24-hour portions. Further details for sampling are given in the Supplemental Data.

For the analysis of ^{14}C radioactivity, expired-air sampling was performed in duplicate while the subject was sitting. Expired-air samples were collected into 4 ml of a trapping solution that contained 2 ml

hyamine hydroxide (1 N) and 2 ml ethanol and thymolphthalein as an indicator. Breath tests were scheduled at the following investigation times: predose, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 72 hours postdose.

Radioactivity Distribution in Rats. Radioactivity concentrations in tissues in rats were quantified from whole-body autoradiograms using a validated image analysis system. The legs, tail, and whiskers were trimmed off, and each frozen carcass was set in a block of frozen aqueous 2% (w/v) carboxymethylcellulose. The block was mounted onto the stage of a CM3600 cryomicrotome maintained at about -20°C [Leica Microsystems (UK) Ltd.], and sagittal sections (nominal thickness 30 μm) were obtained at five levels through the carcass: exorbital lachrymal gland (males) or ovary (females), intraorbital lachrymal gland, harderian gland and adrenal gland, thyroid, brain, and spinal cord. The sections, mounted on Invisible Tape (Supapak), were freeze-dried in a Lyolab B freeze-drier (Life Science Laboratories Ltd.) and placed in contact with FUJI imaging plates (type BAS-MS; Raytek Scientific Ltd.). The ^{14}C blood standards of appropriate activity (also sectioned at a nominal thickness of 30 μm) were placed in contact with all imaging plates. After exposure in a refrigerated, copper-lined, lead exposure box for 4 days, the imaging plates were processed using a FUJI FLA-5000 radioluminography system (Raytek Scientific Ltd.) at a scanning resolution of 100 μm . Electronic images were analyzed using a computer-based image analysis package (Seescan Densitometry software; LabLogic Ltd., Sheffield). The carbon-14 standards included with each autoradiogram were used to construct calibration lines over a range of radioactivity concentrations.

Radioactivity Analysis. For assessment of the excretion balance in rats, radioactivity was determined in urine, feces, cage washings (aqueous and organic), and carcasses. Volumes and/or weights of biologic samples were measured as appropriate. Dried feces homogenates, cage debris, and carcass aliquots were combusted in oxygen using a Packard Sample Oxidizer. The carbon-14 combusted products were

TABLE 2
Apparent paracellular permeability of [^{14}C]imeglimin and reference substance [^3H] cimetidine in Caco-2 cell monolayers (TC7-clone)

Substrate	Concentration (μM)	$P_{\text{app,A-B}}$ ($10^{-6}\text{ cm}^2\text{ s}^{-1}$)	$P_{\text{app,A-B(EGTA)}}$ ($10^{-6}\text{ cm}^2\text{ s}^{-1}$)	Fparacell (%)
Imeglimin	3	2.54	4.91	48.3
	30	1.69	4.68	64.0
	300	0.720	5.13	86.0
Cimetidine	10	0.132	2.92	95.5

Fparacell, contribution of the paracellular permeation to the overall permeation under the in vitro conditions of the model; P_{app} , apparent permeability.

TABLE 3

Bidirectional permeability efflux ratio values for imeglimin across Caco-2 cell monolayers (TC7-clone) in the presence of BCRP (novobiocin) inhibitor

Substrate	Concentration (μM)	$P_{\text{app},A \rightarrow B}$ ($10^{-6} \text{ cm} \cdot \text{s}^{-1}$)	$P_{\text{app},B \rightarrow A}$ ($10^{-6} \text{ cm} \cdot \text{s}^{-1}$)	Efflux Ratio
Imeglimin	3000	1.62	1.24	0.76
Novobiocin	30	1.44	0.91	0.63

P_{app} , apparent permeability.

absorbed in Carbo-SorbR and mixed with PermafluorR E+ liquid scintillant prior to LSC. Radioactivity was measured by LSC using Tri-Carb 2300 (Packard, Dreieich) liquid scintillation counters with the capability to compute quench-corrected disintegrations per minute.

In dogs, the total radioactivity content of plasma and each urine and feces pool was measured by LSC using Tri-Carb 2100 (PerkinElmer, Rodgau). Further details are given in the Supplemental Data.

For clinical samples, analysis of total radioactivity in whole blood, plasma, urine, feces, and expired air (including quick counts in urine and feces) was performed using LSC; a Packard Tri-Carb 3100 TR liquid scintillation analyzer (Downers Grove, IL) was used. Further details are given in the Supplemental Data.

Imeglimin Analysis. The concentrations of imeglimin were investigated in plasma and urine from rats (only plasma) and dogs after single low-dose intravenous and oral administration of 5 mg/kg [^{14}C] imeglimin and from humans after single oral 1000-mg dose by liquid chromatography with tandem mass spectrometric detection method (LC-MS/MS).

Thawed plasma samples (25 μl for animals or 50 μl for human aliquot) were transferred to a 96-well plate. Internal standard (D_6 -imeglimin) solution was added, and the plate was vortex-mixed prior to transfer to a Cerex multichannel solid phase extraction system. The carboxylic acid solid phase extraction plate (50 mg) was conditioned with methanol: 20 mM sodium bicarbonate solution (90:10 v/v, 0.5 ml) followed by addition of 20 mM sodium bicarbonate solution (0.5 ml). Approximately 550 μl or 1 ml of the diluted sample was transferred to the preconditioned cartridge. The sample was then passed through the cartridge, which was subsequently washed with methanol: 20 mM sodium bicarbonate solution (90:10 v/v) (0.5 ml), followed by addition of 10 mM ammonium acetate (0.5 ml). The sample was then eluted into

a fresh 96-well polypropylene collection plate with water/acetonitrile/trifluoroacetic acid (10:90:0.5 v/v/v) (0.5 ml) prior to being submitted for LC-MS/MS analysis. Concentrations of imeglimin in calibration standards, quality control samples, and study samples were determined using least-squares linear regression with the reciprocal of the concentration ($1/x$) as weighting.

Urine samples (20- μl aliquot) were transferred into 20-ml polypropylene scintillation vials, and 100 μl of internal standard solution (20 $\mu\text{g}/\text{ml}$ in water) was added. Mobile phase (8 ml) [10 mM ammonium acetate (native pH)/acetonitrile (20:80) v/v] was then added to each tube; the samples were capped and vortex-mixed, and 10 μl of each sample was aliquoted into a 96-well plate. Mobile phase (490 μl) was added to all wells, and the samples were capped and vortex-mixed again prior to submission for LC-MS/MS analysis. Concentrations of imeglimin in calibration standards, quality control samples, and study samples were determined using least-squares linear regression with the reciprocal of the concentration ($1/x$) as weighting. The blank sample containing internal standard was not used in the regression equation. All data are expressed as hydrochloride salt.

Metabolite Profiling. The metabolite pattern in rat and dog in plasma was investigated after single high-dose oral administration of 100 mg/kg [^{14}C] imeglimin. Metabolite profiling and identification was performed using liquid chromatography with radiodetection and LC-MS/MS techniques.

To identify the structures of the major metabolites in plasma and to support an exact assignment of eluting peaks to metabolite structures, a method using high-performance liquid chromatography, radiometry, and mass spectrometry was used. Structure identification was supported by comparison of retention times and fragmentation spectra of the unknown metabolites with those of the respective synthetic reference compounds. In humans, structure elucidation of metabolites was performed by time-of-flight mass spectrometry. Details are given in the Supplemental Data.

PK Analysis. PK analysis of total radioactivity and imeglimin in plasma and blood was performed using the noncompartmental pharmacokinetic analysis using validated software (Kinetica version 4.1.1).

In rat and dog studies, the following PK parameters were reported: maximum plasma concentration (C_{max}), time to reach maximal concentration (t_{max}), and area under the plasma concentration-time curve (AUC) from time zero to last measurable concentration ($\text{AUC}_{0-\text{last}}$), AUC from time zero to infinity ($\text{AUC}_{0-\infty}$), terminal elimination half-life ($t_{1/2}$), clearance of drug from plasma (CL), and volume of distribution

Fig. 2. Kinetics of [^{14}C] imeglimin uptake by human OCT1.

Fig. 3. Concentration-dependent uptake of imeglimin (EMD 387008) by hOCT2.

(V_{ss}). The bioavailability and fraction absorbed were determined according to the following equations:

$$\text{Bioavailability (\%)} = \frac{(AUC_{0-\text{last}} \text{ po})_{\text{parent drug}}}{(AUC_{0-\text{last}} \text{ iv})_{\text{parent drug}}} \times \frac{\text{Dose iv}}{\text{Dose po}} \times 100$$

$$\text{fraction absorbed} = \frac{(AUC_{0-\text{last}} \text{ po})_{\text{total radioactivity}}}{(AUC_{0-\text{last}} \text{ iv})_{\text{total radioactivity}}} \times \frac{(\text{Dose iv})_{\text{total radioactivity}}}{(\text{Dose po})_{\text{total radioactivity}}} \times 100$$

For clinical study, the following PK parameters were reported for blood and plasma total radioactivity and plasma imeglimin: $C_{\max}$, $t_{\max}$, $AUC_{0-\text{last}}$, $AUC_{0-\infty}$, $t_{1/2}$, apparent clearance of drug from plasma, and apparent volume of distribution (V_{ss}/F).

For each collection interval time, the cumulative amount excreted in urine was calculated as the product of urine concentration and urine volume. CL_R was calculated over the period from 0–48 hours according to the following formula:

$$CL_R = \frac{\text{Amount excreted}}{AUC_{0-\text{last}}}$$

For metabolites, the concentrations of total radioactivity of parent drug and metabolites were measured, and individual $AUC_{0-\text{last}}$ values were calculated; subsequently, the $\%AUC_{TR}$ (AUC of total radioactivity) was calculated using the following formula:

$$\%AUC_{TR} = \frac{AUC_{0-\text{last}} \text{ parent or metabolite}}{AUC_{0-\text{last}} \text{ total radioactivity}} \times 100$$

Results

In Vitro Investigations

In Vitro Permeability Studies. At all concentrations investigated (1–1000 μM), the permeability in the apical-to-basal (A-B) direction (ranging from 5.09 to $0.486 \times 10^{-6} \text{ cm} \cdot \text{s}^{-1}$) was significantly higher than in the basal-to-apical (B-A) direction (ranging from 0.910 to $0.397 \times 10^{-6} \text{ cm} \cdot \text{s}^{-1}$), indicating an active uptake transport process. Results are presented in Table 1. This is supported by the finding that permeability coefficients in the A-B direction declined with increasing concentrations, probably because of saturation of the A-B uptake transport system, whereas permeability coefficients in the B-A direction remained relatively constant. Permeability of imeglimin through Caco-2 cell monolayers (TC7-clone) is 3- to 30-fold lower compared with the high-permeability drug testosterone. Depending on the drug's concentration and integrity of the tight junctions, imeglimin may be classified as an intermediate-permeability drug. Imeglimin was not a P-gp substrate, as the ratio of apparent permeability of B-A versus the A-B direction was lower than 2 (MHLW, 2018, CDER, 2020b). An uptake active transport process in the A-B direction was observed. Using the specific OCT inhibitor decynium, this process was identified as being mediated at least partly by OCT. Imeglimin's apparent permeability A-B was decreased by around 30% in the presence of the OCT inhibitor decynium. Results are presented in Table 1.

The ability of imeglimin to be absorbed utilizing a paracellular permeation processes was investigated in Caco-2/TC7 cell monolayers in the absence and presence of 2 mM EGTA using radiolabeled imeglimin (3, 30, and 300 μM). Permeability of cimetidine increased by a factor of about 20 in the presence of EGTA, indicating that the paracellular route contributes 95% of the overall permeation of this compound in the artificial conditions of the in vitro Caco-2 model.

TABLE 4

Accumulation of imeglimin in MATE1, MATE2-K, OAT1, and OAT3 transporter-expressing and control cells measured and inhibition follow-up of MATE1 and MATE2-K

Compound	Expressing Cells	Concentration (mM)	Incubation Time (min)	Accumulation (pmol/mg protein)			
				In Expressing Cells	In Control Cells	Active Transport	Fold Accumulation
Imeglimin	MATE1	1	2	1198	1060	138	1.13
			20	4722	3300	1422	1.43
		0.1	2	183	53.1	130	3.45
			20	2178	351	1828	6.21
Imeglimin	MATE1	0.1	20	7911	1553	6358	5.09
Pyrimethamine ^a		0.001	20	2328	1953	374	1.19
Imeglimin	MATE2-K	1	2	4153	935	3218	4.44
			20	6720	2137	4583	3.14
		0.1	2	571	54.4	517	10.5
			20	1818	234	1585	7.78
Imeglimin	MATE2-K	0.1	2	3570	351	3219	10.2
Pyrimethamine ^a		0.001	2	883	468	415	1.89
Imeglimin	OAT1	1	2	1474	841	633	1.75
		0.1	2	120	193	−73.6	0.62
Imeglimin	OAT3	1	2	1816	1148	668	1.58
		0.1	2	25.3	29.9	−4.58	0.85

^aInhibitor.

TABLE 5
Inhibitory effect of imeglimin on specific activities of cytochrome P450 isoforms in human liver microsome (remaining activity)

Cytochrome P450 Isoform	Metabolic Reaction	Remaining Activity (%)				
		200 μ M	500 μ M	1000 μ M	3000 μ M	6000 μ M
CYP1A2	Phenacetin <i>O</i> -deethylation	91.7	85.3	74.7	— ^a	—
CYP2B6	Bupropion hydroxylation	95.7	97.3	95.2	—	—
CYP2C8	Paclitaxel 6 α -hydroxylation	98.1	100.9	97.2	—	—
CYP2C9	Diclofenac 4'-hydroxylation	97.3	99.0	96.7	—	—
CYP2C19	(S)-Mephenytoin 4'-hydroxylation	96.2	94.1	91.9	—	—
CYP2D6	Bufuralol 1'-hydroxylation	92.9	87.9	84.2	—	—
CYP3A4	Midazolam 1'-hydroxylation	—	—	89.2	81.4	67.9
CYP3A4	Testosterone 6 β -hydroxylation	—	—	90.3	77.8	61.1

^aNot performed

The apparent permeability of imeglimin increased significantly in a concentration-dependent manner in the presence of EGTA (from 2-fold at 3 μ M to 7-fold at 300 μ M). The contribution of the paracellular route increased with the imeglimin concentration employed and amounted up to 86%. With tight junctions opened, the permeability is even higher (two times) than for cimetidine, classifying imeglimin as an intermediate-permeability drug. Results are presented in Table 2.

Imeglimin was not a BCRP substrate, as the efflux ratios were lower than 2 (MHLW, 2018, CDER, 2020b). Indeed, the efflux ratio values were very similar in the absence (0.76) and in the presence of the BCRP inhibitor novobiocin (0.63). Results are presented in Table 3.

In Vitro Metabolism in Human and Rat Subcellular Fractions. No evidence of metabolism of imeglimin was observed in any of the subcellular fractions derived from tissues of either human or Wistar rat—namely, liver, lung, intestines, or kidney S9 and microsomes. This was demonstrated by the absence of NADPH- and incubation-dependent new chromatographic peaks without disappearance of imeglimin. The results clearly show the absence of any metabolic lability of imeglimin when exposed to rat or human in vitro systems—not only mimicking hepatic cytochrome P450 metabolism but also covering the whole range of tissues and organs that contain appreciable amounts of cytochrome P450 isoforms known to metabolize xenobiotics.

Binding to Plasma Proteins and Blood Cell Partitioning. At 1, 10, and 100 μ M, plasma protein binding of [¹⁴C]imeglimin was low in all species. Mean values of bound drug across the concentration range were of 4.8%–8.3%, 5.7%–6.8%, and 5.3%–6.4% in rat, dog, and human,

respectively. No concentration-dependent effects on plasma protein binding were observed.

Blood cell partition coefficients of [¹⁴C]imeglimin indicate that the drug was similar across the concentration range, with mean values of 1.05, 0.27, and 0.48 in rat, dog, and human, respectively. Thus, imeglimin was equally distributed between blood cells and plasma in rat, whereas in dog and human, plasma concentrations were 2 to 3 times higher than in blood cells. There was no noticeable degradation of [¹⁴C] imeglimin in whole blood of any species after incubation at 37°C up to 4 hours.

Substrate of Hepatic or Renal Transporters. In the time course study, the uptake amount of [¹⁴C]imeglimin in OCT1/HEK293 cells was significantly higher than that in Mock/HEK293 cells, indicating that imeglimin is a substrate of OCT1. In the kinetic study, concentration-dependent imeglimin uptake by OCT1 was observed; Michaelis-Menten parameters were determined with a K_m of 1130 μ M and a V_{max} of 16,500 pmol/mg protein per minute (Fig. 2). The calculated intrinsic uptake clearance of imeglimin by human OCT1 was 14.6 μ L/mg protein per minute. The transport of [¹⁴C]imeglimin in hOCT2-expressing cells was significantly increased compared with vector-transfected control cells. This uptake was time-dependent and saturable, with a K_m of 41.1 μ M and a V_{max} of 1490 pmol/min per milligram protein. A clearance of 36 μ L/min per milligram protein was calculated (Fig. 3).

The accumulation of imeglimin was similar in the OAT1 and OAT3 transporter-expressing and the control cells; the transporter-specific fold accumulations were lower than 2 (MHLW, 2018, CDER, 2020b), indicating that there was no active accumulation of imeglimin under all conditions tested (Table 4).

TABLE 6
Fold induction of CYP1A2, CYP2B6, and CYP3A4 mRNA by imeglimin and by control inducers

Cytochrome P450 Isoform	Compound	Concentration (μ M)	Fold Induction		
			Donor 1	Donor 2	Donor 3
CYP1A2	Imeglimin	20	0.93	1.0	1.2
		60	1.2	1.2	1.5
		120	1.3	1.2	1.8
CYP2B6	Omeprazole	50	36	13	11
	Imeglimin	20	0.89	0.93	0.97
		60	0.89	0.87	0.87
		120	0.74	0.70	0.76
CYP3A4	Phenobarbital	2000	6.4	12	7.9
	Imeglimin	20	0.98	0.97	1.1
		60	1	0.82	1.2
		120	0.97	0.55	1.0
	Rifampicin	25	22	45	32

TABLE 7

Effect of imeglimin at 1 mM on the MATE1 (metformin)-, MATE2-K (metformin)-, OAT1 (*p*-aminohippuric acid)-, and OAT3 (E3S)-mediated transport of substrate measured in the uptake transporter inhibition assay

Transporter	Transporter-Specific Accumulation (cpm)	Relative Transporter-Specific Accumulation (% of Control)	Relative Inhibition (%)
MATE1	9.33	3.22	97
MATE2-K	186	61.6	38
OAT1	2659	94.2	6
OAT3	3160	90.7	9

The highest fold accumulation (6.21) of imeglimin occurred with MATE1; this was observed at a 0.1 mM concentration of imeglimin applying a 20-minute incubation period (Table 4). For MATE2-K, the highest fold accumulation value was 10.50 at a 0.1 mM concentration and 2-minute incubation time (Table 4). To confirm the transporter specificity of these interactions, the assays were repeated in the absence and presence of specific inhibitors of the MATE1 and MATE2-K transporters. In these follow-up inhibition assays, the fold accumulations observed at 0.1 mM and 20 minutes (MATE1) and at 0.1 mM and 2 minutes (MATE2-K) were reproducible, and the inhibitors decreased the transporter-specific accumulation of imeglimin from 5.09-fold to 1.19-fold with MATE1 and from 10.2-fold to 1.89-fold with MATE2-K, verifying the interactions (Table 4). Therefore, imeglimin was confirmed to be a substrate of OCT1, OCT2, MATE1, and MATE2-K transporters.

In Vitro Assessment of DDI Potential

Cytochrome P450 Inhibition Studies. The results of these studies indicated that imeglimin does not appear to cause any substantial direct inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5. The IC_{50} values for these enzymes were greater than the highest concentration of imeglimin studied (up to 1000 μ M and up to 6000 μ M for CYP3A4). Results are shown in Table 5. Furthermore, there was little or no evidence that imeglimin has the potential to cause time-dependent inhibition of any of the cytochrome P450 enzymes evaluated (data not shown).

Cytochrome P450 Induction Studies. Positive control inducers (50 μ M omeprazole, 2000 μ M phenobarbital, and 25 μ M rifampicin) demonstrated 11- to 36-fold increases of CYP1A2 mRNA, 6.4- to 12-fold increases of CYP2B6 mRNA, and 22- to 45-fold increases of CYP3A4 mRNA, respectively, indicating that the induction assays for these cytochrome P450 isoforms were performed appropriately. Under the same conditions, the induction potential of imeglimin was evaluable.

The influence of imeglimin on CYP1A2 mRNA expression was assessed in three donors; fold inductions were 0.93–1.2, 1.2–1.5, and 1.2–1.8, respectively, at 20, 60, and 120 μ M. With CYP2B6 mRNA expression in the three donors, the fold inductions were 0.89–0.97, 0.87–0.89, and 0.70–0.76, respectively, at 20, 60, and 120 μ M. With CYP3A4 mRNA expression in the three donors, the fold inductions were 0.97–1.1, 0.82–1.2, and 0.55–1.0, respectively, at 20, 60, and 120 μ M. All results are presented in Table 6. In conclusion, imeglimin

showed no induction potential on the mRNA expression of CYP1A2, CYP2B6, and CYP3A4 up to 120 μ M, as the fold inductions were lower than 2 (MHLW, 2018, CDER, 2020b).

Transporter Inhibition Study. The in vitro interaction potential of imeglimin with human MATE1, MATE2-K, OAT1, OAT3, OATP1B1, and OATP1B3 transporters was tested at 0.1 and 1 mM concentrations. In these experiments, imeglimin did not influence the OAT1- and OAT3-mediated probe substrate accumulation at the applied concentrations, up to 1 mM; relative inhibition of 6% and 9%, respectively, was measured, as shown in Table 7. However, the compound inhibited MATE1- and MATE2-K-mediated probe substrate transport, with a maximum inhibition of 97% and 38%, respectively, observed at the 1 mM imeglimin concentration. No relevant inhibition of MATE2-K by imeglimin was considered, as the IC_{50} was above 1 mM. For MATE1, the calculated IC_{50} value was 19.24 μ M. Results are presented in Table 7.

Imeglimin did not influence OATP1B1- and OATP1B3-mediated probe substrate uptake, with inhibition values of only 5.6% and –2.5%, respectively (Table 8). At imeglimin concentrations of 1, 2, and 3 mM, the remaining activity of [3 H]digoxin transport by P-gp was 87.0%, 71.8%, and 91.1%, respectively (13.0%, 28.2%, and 8.9% inhibition, respectively). These results indicate that imeglimin does not inhibit P-gp-mediated [3 H]digoxin transport (up to 3 mM). Results are presented in Table 9.

No inhibition of BCRP-mediated probe substrate transport by imeglimin was observed. The relative inhibition was 3% and 7% with 3 and 0.3 μ M of imeglimin, respectively (Table 10).

The inhibition potency of imeglimin toward hOCT1 was determined using [3 H]MPP+ as a substrate, and an IC_{50} value of 154 μ M was calculated. Results are shown in Fig. 4.

The inhibition potency of imeglimin toward hOCT2 was determined using [3 H]MPP+ as a substrate, and an IC_{50} value of 146 μ M was calculated. Results are shown in Fig. 5.

Animal and Human 14 C ADME Studies

ADME, PK, Metabolism, and Tissue Distribution in Rats. Imeglimin and total radioactivity PK parameters are shown in Table 11. After single intravenous administration of 5 mg/kg (low dose), plasma concentrations of total radioactivity declined rapidly, with a half-life of 2.9 hours (Fig. 6A). The V_{ss} determined after intravenous injection was

TABLE 8

Inhibitory effect of imeglimin at 1000 μ M on the OATP1B1- and OATP1B3-mediated transport of substrate (estradiol-17 β -D-glucuronide) measured in the uptake transporter inhibition assay

Transporter	OATP/ HEK293 Cells	Mock/ HEK293 Cells	Mediated Uptake	Remaining Activity (%)	Inhibition (%)
OATP1B1	1.93	0.0853	1.84	94.4	5.6
OATP1B3	0.822	0.0853	0.737	102.5	–2.5

TABLE 9
Inhibitory effect of imeglimin on [³H]digoxin transport by human P-gp

Compound	Concentration (μM)	P-gp/LLC-PK1 Cells			Mock/LLC-PK1 cells			NFR	Remaining Activity (%)	Inhibition (%)
		P _{app,A-B} (10 ⁻⁶ cm·s ⁻¹)	P _{app,A-B} (10 ⁻⁶ cm·s ⁻¹)	FR	P _{app,A-B} (10 ⁻⁶ cm·s ⁻¹)	P _{app,A-B} (10 ⁻⁶ cm·s ⁻¹)	FR			
Imeglimin	1000	0.560	12.4	22.1	1.98	5.04	2.28	9.44	87.0	13.0
	2000	0.609	11.9	19.5	1.97	4.64	2.34	7.96	71.8	28.2
	3000	0.527	12.8	24.3	2.02	4.82	2.45	9.84	91.1	8.9

FR, flux ratio; NFR, net flux ratio; P_{app}, apparent permeability.

2.1 l/kg. The CL was medium to low (1.2 l/h per kilogram). After oral administration, peak concentrations of both parent drug and total radioactivity in plasma were reached between 1 and 2 hours postdose (Fig. 7A). After administration of a high oral dose (100 mg/kg), the peak plasma concentration was reached after 2 hours, and plasma concentrations plateaued up to 8 hours (data not shown).

The absolute bioavailability, calculated from the ratio of the AUCs of the parent drug after oral versus intravenous administration of the low dose, was 30%; the extent of absorption from the gastrointestinal tract, calculated from the ratio of the AUCs of total radioactivity, was 62%.

The distribution of radioactivity into tissues was similar, whatever the strain and the sex of animals. However, in intravenously dosed female animals, radioactivity concentrations were higher after intravenous administration than after oral administration, with the ratio of concentrations being over 2 for more than half of the tissues. Tissues with tissue-to-blood ratios above 3 included the small intestine mucosa (even after intravenous administration), lateral nasal gland, salivary glands, kidney medulla, brown fat, myocardium, thymus, and liver. Lower concentrations of radioactivity were associated with white fat, the central nervous system (because of very limited transfer across the blood-brain barrier), the lens of the eye, and most reproductive organs. Most tissues no longer contained quantifiable concentrations of radioactivity 24 hours after dosing. There was a weak and reversible binding of drug-related radioactivity to melanin. Whole-body autoradiograms of a female albino rat 1 hour after a single oral and intravenous dose at 5 mg/kg are presented in Supplemental Figs. 1 and 2, respectively. Concentrations of radioactivity in the tissues of male and female albino rats after single oral and intravenous (female only) doses are presented in Supplemental Table 1.

The extent of metabolism ranged from 70% at the dose of 5 mg/kg to 30% at the dose of 100 mg/kg based on the ratio of total radioactivity exposure over the imeglimin exposure (Tables 11 and 12). The metabolites of imeglimin are presented as percentage of total plasma radioactivity AUC after oral administration of 100 mg/kg [¹⁴C] imeglimin in Table 12, and the metabolic pathway is presented in Fig. 8.

In pooled plasma samples from male rats, the predominant radioactive constituent within 24 hours was unchanged imeglimin (Fig. 9A). Besides the parent drug, essentially two main circulating radioactive metabolites—i.e., EMD 647302, produced through *N*-demethylation of imeglimin, and EMD 601811, generated by oxidative aromatization

together with *N*-demethylation—were detected. Only at 8 hours after dosing, two minor circulating radioactive components—i.e., EMD 27355, produced by oxidative aromatization together with di-*N*-demethylation, and M155-3, most probably formed by *N*-demethylation together with oxidation of the *N*-methyl group—were detected. The structure of M155-3 was tentatively assigned. In addition, EMD 90076, formed by oxidative aromatization of imeglimin, was below the limit of radioactive peak detection (1% of plasma or urine sample radioactivity) and could be observed in trace amounts only by mass spectrometry. Mass spectral data and representative product ion mass spectra of imeglimin and metabolites are shown in Supplemental Table 2 and Supplemental Figs. 3–9.

After an intravenous administration of [¹⁴C]imeglimin, radioactivity was rapidly eliminated; by 48 hours postdose, excretion was essentially complete (Fig. 10A). The principal route of elimination was in urine, with approximately 85% of the radioactivity being recovered over 48 hours; 65.8% of the dose was already excreted in urine at 8 hours postadministration, showing a rapid excretion of radioactivity. Fecal elimination accounted only for approximately 3% of the administered dose, suggesting limited biliary excretion and/or intestinal secretion. In contrast, after an oral administration of [¹⁴C]imeglimin, the major route of elimination was via feces, accounting for 52% of the dose, with urinary excretion accounting for 38%. As with the intravenous route, elimination of radioactivity was rapid, with approximately 75% and 85% of the administered dose being eliminated by 8 and 24 hours postdose, respectively.

Overall, recoveries of radioactivity were acceptable (96% for intravenous and 92% for oral administrations).

ADME, PK, and Metabolism in Dogs. Imeglimin and total radioactivity PK parameters after oral and intravenous administrations are shown in Table 11.

After a single intravenous dose, plasma levels of total radioactivity and imeglimin showed a multiexponential decline. Plasma concentrations declined rapidly to approximately 20% of the maximal concentration already by 1 hour after dosing and were below the limit of detection at 48 hours postdosing (Fig. 6B). The V_{ss} at steady state was determined after intravenous injection and was 1.95 l/kg, which indicates extensive uptake by organs and tissues. The CL was low (0.65 l/h per kilogram).

No significant differences between parent drug and ¹⁴C radioactivity for plasma concentration versus time profiles were observed.

TABLE 10
Effect of imeglimin on the BCRP-mediated transport E3S measured in the vesicular transport inhibition assay

Compound	Concentration (mM)	ATP-Dependent Transport (pmol/mg per minute)	Relative ATP-Dependent Transport (% of Control)	Relative Inhibition (%)
Imeglimin	3	900	96.7	3
	0.3	866	93.1	7
Ko134	0.001	7.82	0.99	99

Fig. 4. Concentration-dependent inhibition of specific MPP+ uptake by imeglimin in hOCT1-expressing HEK cells.

After a single oral administration and based on the data after intravenous administration, the mean extent of absorption of total radioactivity was found to be 82.4%, and the absolute bioavailability was found to be 75.8% (Fig. 7B).

The metabolites of imeglimin were quantified and identified in plasma, urine, and feces after oral administration of 100 mg/kg [^{14}C] imeglimin. Mass spectral data and representative product ion mass spectra of imeglimin and metabolites are shown in Supplemental Table 2 and Supplemental Figs. 3–9.

The metabolites of imeglimin are presented as percentage of total plasma radioactivity $\text{AUC}_{0-6 \text{ h}}$ in Table 12, and the metabolic pathway is presented in Fig. 8. The major circulating radioactive component in plasma was imeglimin after oral administration (Fig. 9B). *N*-demethylation of imeglimin formed the major circulating metabolite, EMD 647302. Oxidative aromatization and di-*N*-demethylation of imeglimin formed the minor metabolite, EMD 27355, which was observed in the 6-hour sample only.

The major radioactive component in urine was unchanged imeglimin, representing approximately 43% of the administered dose within 48 hours and 80% of the total radioactivity excreted in urine (Supplemental Fig. 10A). The main radioactive metabolite was EMD 647302, formed by *N*-demethylation, representing 8% of the administered dose (15% of the radioactivity excreted in urine) within 48 hours. Two minor metabolites—namely, M127 (formed by di-*N*-demethylation) and EMD 27355 (formed by oxidative aromatization together with di-*N*-demethylation)—accounted for 2% and 1% of the administered dose, respectively.

The major radiolabeled component in fecal homogenates was unchanged imeglimin, representing 40% of the administered dose and 99% of the total radioactivity excreted in feces (Supplemental Fig. 10A). Besides unchanged drug, only the metabolite EMD 647302 (*N*-demethylation of imeglimin) appeared 24 hours after dosing in low quantities (less than 1% of the dose).

The mean total recovery of ^{14}C radioactivity was 96.2%, 92.2%, and 88.2% of the administered dose for intravenous, low oral, and high oral doses, respectively (Fig. 10B). For both routes and doses, the major route of elimination of the radioactivity was via the kidneys (95.2%, 76.6%, and 55.1% of the administered dose in urine after intravenous, oral low-dose, and oral high-dose administrations of [^{14}C]imeglimin, respectively). The excretion in feces accounted for 1.0%, 16%, and 33% of the

Fig. 5. Concentration-dependent inhibition of specific MPP+ uptake by imeglimin in hOCT2-expressing HEK cells.

administered dose for the period from 0–120 hours after intravenous, low-dose, and high-dose oral administrations, respectively. The lower renal excretion after oral high dose compared with the low dose, together with the corresponding higher portion excreted in feces, may be due to saturation of intestinal absorption. The ^{14}C radioactivity was rapidly eliminated from the organism: within 24 hours, 91.4%, 85.5%, and 80.3% of the administered dose has been found in urine and feces after intravenous, low-dose oral, and high-dose oral administrations, respectively. In total, 70.5% of the dose administered was already excreted in urine 8 hours post-intravenous administration, whereas only 40.1% of the dose or 52% of the total amount excreted was measured in urine 8 hours post-oral administration. This suggests that the elimination was slower after oral administration than after intravenous administration or that the rate of absorption controls the rate of elimination.

Within 24 hours, the mean total recovery of parent drug in urine was about 57% and 78% after single low-dose oral and intravenous administration, respectively. Mean CL_R of parent drug was 0.51 l/h per kilogram, close to total body clearance (0.65 l/h per kilogram), confirming that metabolic clearance of imeglimin was low.

ADME, PK, and Metabolism in Humans. Imeglimin and total radioactivity PK parameters after single oral administration of 1000 mg [^{14}C]imeglimin, given as a solution, are shown in Table 11.

The concentration-time profiles showed that shortly after administration, the concentrations of ^{14}C radioactivity in plasma and whole blood and the concentrations of imeglimin in plasma increased rapidly. The C_{max} was reached between 1.5 and 4 hours after dosing, with a median t_{max} at 3.5 hours. Absorption can be prolonged up to 4 hours. The ^{14}C radioactivity in plasma and whole blood declined steadily after C_{max} up to 24 hours postdose, after which all individual concentrations were below the limit of quantification. Imeglimin plasma concentrations decreased in a biphasic manner, characterized by an initial rapid drop from C_{max} up to 24 hours postdose and followed by a slower decline up to 72 hours postdose (Fig. 7, C and D). The mean imeglimin apparent $t_{1/2}$ was about 13 hours. The V_{ss}/F was high (1422 l).

The mean C_{max} and AUC values for ^{14}C radioactivity in plasma were higher than plasma values for imeglimin.

The geometric mean blood-cell-to-plasma ratio for ^{14}C radioactivity was approximately 0.45 from 0.5 hours postdose to 4 hours postdose. From 4 hours postdose onward, the ratio increased to about 2.60 at 16 hours postdose. By 36 hours postdose, all individual concentrations of ^{14}C radioactivity in plasma and whole blood were below the limit of quantification.

TABLE 11

Imeglimin and ^{14}C radioactivity geometric mean (CV%) PK parameters after oral and intravenous administration of a single dose of 5 mg/kg or 100 mg/kg of [^{14}C]imeglimin in rats and dogs and after oral administration of a single dose of 1000 mg [^{14}C]imeglimin in humans

Species	Dose	Route	Analyte	C_{max} (ng-eq/mL or ng/mL)	t_{max} (h)	$\text{AUC}_{0-\text{last}}$ (ng-eq/h/mL or ng-h/mL)	$\text{AUC}_{0-\infty}$ (ng-eq/h/mL or ng-h/mL)	$t_{1/2}$ (h)	CL/F (L/h) or CL (L/h/kg)	V_{ss}/F (L) or V_{ss} (L/kg)
Human	1000 mg	Oral	^{14}C radioactivity plasma	1769 (14)	3.5 (1.5–4.0)	14,977 (16)	15,933 (17)	3.96 (42)	62.4 (16)	356 (32)
			^{14}C radioactivity blood	1358 (10)	4.0 (1.0–4.0)	13,412 (20)	16,233 (15)	6.16 (8)	61.3 (14)	544 (10)
Rat	5 mg/kg	Intravenous	Imeglimin	1432 (14)	3.5 (1.5–4.0)	12,223 (17)	12,604 (18)	13.0 (25)	75.7 (17)	1422 (22)
			^{14}C radioactivity	6016	NA	4243	4320	2.9	1.16	2.07
Dog	5 mg/kg	Oral	^{14}C radioactivity	5924	NA	6972	7233	5.6	NC	NC
			Imeglimin	404	1.0	1220	1299	2.3	NC	NC
	5 mg/kg	Intravenous	^{14}C radioactivity	606	1.0	4247	4481	NC	NC	NC
			Imeglimin	12,593 (21)	NA	9608 (8)	9777 (8)	5.74 (9)	0.645 (9)	1.94 (14)
	5 mg/kg	Oral	^{14}C radioactivity	12,636 (10)	NA	11,749 (7)	12,451 (6)	6.90 (5)	0.506 (7)	2.35 (8)
			Imeglimin	1428 (43)	1.0 (1.0–2.0)	7190 (28)	NC	NC	NC	NC
			^{14}C radioactivity	1576 (34)	1.0 (1.0–2.0)	9615 (23)	NC	NC	NC	NC

CL/F, apparent clearance of drug from plasma, NC, Not calculated, NA, Not applicable.

Fig. 6. Imeglimin and ^{14}C radioactivity arithmetic mean concentrations after intravenous administration of a single dose of 5 mg/kg [^{14}C]imeglimin in rats (A) and dogs (B).

The metabolites of imeglimin are presented as percentage of total plasma radioactivity AUC (0–12 hours) in Table 12, and the metabolic pathway is presented in Fig. 8. Mass spectral data and representative product ion mass spectra of imeglimin and metabolites are shown in Supplemental Table 2 and Supplemental Figs. 3–9.

The major circulating radioactive component in pooled plasma was unchanged imeglimin, representing 93% of the AUC within 12 hours after oral administration (Fig. 9C). Two further circulating radioactive components—i.e., EMD 601811, formed by oxidative aromatization and *N*-demethylation, and EMD 27355, produced by oxidative aromatization and di-*N*-demethylation—were found in much lower quantities (5% and 2%, respectively). In addition, other drug-related compounds—i.e., EMD 647302, formed by *N*-demethylation, and EMD 90076, which is a product of oxidative aromatization of imeglimin—were below the limit of radioactive peak detection and could only be detected by mass spectrometry. Based on the limit of quantification for radioactive peak detection (1% of the total radioactive peak area) for calculation of the plasma levels, these components would account for less than 1% of the AUC of total radioactivity.

To assess the excretion kinetics, ^{14}C radioactivity was determined in urine, feces, and expired air. No ^{14}C radioactivity was detected in expired-air samples.

Excretion in the urine and feces was roughly equally distributed (Fig. 10C). Consequently, renal excretion was between 32% and 55% and fecal excretion was between 44% and 66% of the administered radioactive dose.

Fig. 7. Imeglimin and ^{14}C radioactivity mean concentrations after oral administration of a single dose of 5 mg/kg ^{14}C imeglimin in rats (A) and dogs (B) and after oral administration of a single dose of 1000 mg ^{14}C imeglimin in humans in linear and semilogarithmic scale (C and D).

In urine, imeglimin was almost exclusively excreted unchanged, accounting for 42.7% of the dose administered and for 97% of the total radioactivity excreted in urine. Besides the parent drug, two minor urinary radioactive metabolites were observed—namely, EMD 27355 and EMD 647302, representing 0.3% and 0.7% of the administered dose, respectively (Supplemental Fig. 10B). The arithmetic mean CL_R was 35.4 l/h. Imeglimin CL_R was higher than the creatinine clearance (6.108 l/h), indicating that imeglimin was actively secreted into urine.

In feces, ^{14}C radioactivity was excreted almost exclusively as unchanged drug, representing 99% of the recovered fecal radioactivity (Supplemental Fig. 10B).

Therefore, approximately 98% of the radioactive dose was excreted as unchanged compound in urine and feces.

Discussion

To support the development of new chemical drug, the assessment of its absorption, distribution, metabolism, and excretion, as well as the identification of enzymes and transporters involved in pharmacokinetics of the compound, are key results needed to understand the potential risk of drug-drug interactions. In this context, imeglimin pharmacokinetics

was assessed through several in vitro and in vivo experiments in animals and humans.

After imeglimin oral administration in animals, t_{max} values indicated a medium rate of absorption, with averages ranging from 2 to 3 hours across species. In rats, after oral administration of 100 mg/kg, concentrations can plateau until 8 hours when dose increases, indicating a prolongation of absorption (Fig. 7). Imeglimin was well absorbed from the gastrointestinal tract (62% and 82% in rats and dogs, respectively). The oral bioavailability was moderate to high (30% and 76% in rats and dogs, respectively). In humans, the concentration-time profiles showed that shortly after administration, ^{14}C radioactivity in plasma and whole blood and the concentrations of imeglimin in plasma increased rapidly (Fig. 7C). The C_{max} was reached between 1.5 and 4 hours after dosing, with a median t_{max} at 3.5 hours (Table 11).

Permeability of imeglimin was 3- to 30-fold lower compared with the high-permeability drug testosterone, depending on the drug's concentration and integrity of the tight junctions, thus classifying imeglimin as an intermediate-permeability drug (Table 1). Imeglimin permeability in the A-B direction was significantly higher than in the B-A direction, indicating an active transport process, which has been shown to be at least partly mediated through OCT. The permeability coefficients in the

TABLE 12

Metabolites of imeglimin as percentage of total plasma radioactivity AUC (0–12 h) and as percentage of dose administered in urine and feces after single oral administration of ^{14}C imeglimin to rat, dog, and humans

Matrix	Rat (100 mg/kg)	Dog (100 mg/kg)			Human (1000 mg)		
	Plasma	Plasma	Urine	Feces	Plasma	Urine	Feces
EMD27355	NC	NC	2%	— ^a	2%	0.3%	—
M155-3	NC	—	—	—	—	—	—
EMD647302	3.3%	10%	8%	<1%	NC	0.7%	—
EMD601811	13%	—	—	—	5%	—	—
EMD90076	—	—	—	—	NC	—	—
Imeglimin	69%	86%	43%	40%	93%	42.7%	55.4%

NC, AUC_{0–12} was not calculated (observed by mass spectrometry only).

^aNot performed

Fig. 8. Metabolite pathway of imeglimin in rats, dogs, and humans. R, D, and H (respectively, rat, dog, and human) denote in which species the metabolite was observed.

*: To be confirmed by NMR

A-B direction were observed to decline with increasing concentrations because of the saturation of the A-B transport system (Table 1). It was also shown that imeglimin was significantly absorbed via the paracellular route. Its permeability was increased from 2- to 7-fold in the presence of EGTA, which loosens tight junctions when imeglimin concentration was raised from 3 to 300 μM (Table 2). Obviously, saturation of the active transport pathway at increasing concentrations of imeglimin directs the small and positively charged drug to the paracellular route to a greater extent.

These observations are in accordance with data from phase I studies showing that the absorption of imeglimin in humans decreased from ~50% to 30% when doses were increased from 100 to 2000 mg (P. Fouqueray et al., submitted manuscript).

Intravenous administration in animals showed a rapid decrease in plasma concentrations, suggesting a rapid and extensive distribution into organs (Fig. 6). This distribution could be facilitated by the low plasma protein binding of ^{14}C imeglimin observed in vitro at 0.1–100 μM .

Indeed, plasma protein binding of ^{14}C imeglimin was low in all species; mean values across the concentration range tested were 4.8%–8.3%, 5.7%–6.8%, and 5.3%–6.4% in rat, dog, and human, respectively. In humans, a time-dependent change in the mean blood-cells-to-plasma ratio of ^{14}C radioactivity was observed. This might be explained by changes in the plasma concentration of ^{14}C radioactivity and equilibration between concentrations in blood cells and plasma. By 36 hours postdose, all individual concentrations of ^{14}C radioactivity in plasma and whole blood were below the lower limit of quantification,

suggesting that there was no long-term binding of ^{14}C radioactivity to red blood cells from that time on.

In vitro experiments show that imeglimin is poorly metabolized in animals and humans and that clearance by metabolism is a minor pathway of elimination.

These results are consistent with in vivo studies. As already indicated by both the high amount of fraction absorbed and good oral bioavailability, imeglimin was metabolized to a low extent, except in rats at low dose. Across all species, the major circulating radioactive component in plasma was unchanged imeglimin, without detection of a unique human metabolite (Fig. 9; Table 12). All metabolites also represented less than 10% of the total exposure, allowing us to conclude there was no disproportionate metabolite exposure. In humans, after oral administration of [^{14}C]imeglimin, unchanged imeglimin represented 93% of plasma total radioactivity.

In humans, the main biotransformations occurred through oxidative aromatization and further N-demethylation (EMD 601811) and oxidative aromatization together with di-N-demethylation (EMD 27355), representing 5% and 2% of the exposure, respectively (Fig. 8; Table 12). Both of these metabolites were also found in the metabolic pattern of dogs and rats.

In animals, after intravenous and oral administration, ^{14}C radioactivity was rapidly eliminated from the organism. For both routes, the major path of elimination of radioactivity was via the kidneys (Fig. 10). The excretion in feces was low after intravenous administration but higher after oral administration. In total, 65.8% and 70.5% of the dose

Fig. 9. Representative radio-high-performance liquid chromatography separation of the radioactive constituents in plasma samples of (A) male Wistar rats (8 hours after dose), (B) female dog (6 hours after dose), (C) male healthy volunteer (6 hours after dose) after single oral administration of [^{14}C]imeglimin.

administered was already excreted in urine 8 hours after intravenous administration in rat and dog, respectively, whereas in dog, only 40.1% of the dose or 52.0% of the total amount excreted in urine was excreted in urine 8 hours after oral administration (Fig. 10B). This suggests that elimination was slower after oral administration than after intravenous administration or that the rate of absorption controls the rate of elimination. In humans, excretion in the urine and feces was roughly equally distributed. Similar to dogs, 64% of the total amount excreted

into urine was excreted 8 hours postadministration. It is anticipated that imeglimin exhibits a “flip-flop” pattern of pharmacokinetics in which the rate of absorption of a drug is slower than its rate of elimination; therefore, the terminal phase of the AUC profile reflects the absorption rate, and the initial rising phase represents the elimination of drug.

Importantly, in humans, approximately 98% of the radioactive dose was excreted as unchanged compound in urine and feces.

In terms of the drug-drug interaction profile, there is little or no evidence suggesting that imeglimin has the potential to cause metabolism-dependent DDI, as imeglimin is poorly metabolized and has no inhibition potential toward CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 (up to 1000 μM for all cytochrome P450s and up to 6000 μM for CYP3A4/5). These concentrations largely covered the maximal clinical plasma concentration observed at steady state, ~ 10 μM , and the imeglimin intestinal concentration potentially leading to an inhibition of intestinal CYP3A4/5 (3 mM). Moreover, imeglimin did not display inducing effects on CYP1A2, CYP2B6, or CYP3A4. Since the highest concentration tested, 120 μM , is about 16-fold higher than C_{max} observed in the clinical ADME study (1432 ng/ml = 7.5 μM), imeglimin can be considered unlikely to induce cytochrome P450 in clinical settings, even if a potential induction of intestinal CYP3A4 cannot be formerly ruled out.

As imeglimin is actively secreted in urine by the kidney, we investigated whether imeglimin was a substrate of renal transporters as OAT1, OAT3, OCT2, MATE1, and MATE2-K. Imeglimin was found to be a substrate of OCT2, MATE1, and MATE2-K uptake transporters and also a liver transporter, OCT1 (Figs. 2 and 3; Table 4), which explains its active transport. In dogs and humans, the decrease in the fraction excreted in urine observed with increasing doses, associated with the increase in fecal excretion (in dogs), could be explained by saturation of intestinal absorption mediated at least partly through OCT transporters. In a clinical DDI study conducted using cimetidine, an inhibitor of OCT1, OCT2, MATE1, and MATE2-K, imeglimin’s exposure increased when cimetidine was coadministered compared with when it was not coadministered, but these increases were not considered as clinically significant, with the magnitude of increase being 1.34-fold and 1.27-fold, respectively, for C_{max} and $\text{AUC}_{0-\text{last}}$. The increase in imeglimin exposure is thought to be mainly mediated by the cimetidine-mediated inhibition of MATE1 renal secretion (Chevalier et al., 2020).

Imeglimin is not a substrate of OAT1 and OAT3 uptake (solute carrier family) transporters. Therefore, no dedicated clinical DDI study was performed (Table 4).

Potential inhibitory effects of imeglimin on uptake of typical substrates for various transporters were evaluated using cells expressing human OCT1, OCT2, MATE1, MATE2-K, OAT1, OAT3, OATP1B1, and OATP1B3 (Figs. 4 and 5; Tables 7–9). Imeglimin did not show any inhibitory effect on MATE2-K, OAT1, OAT3, OATP1B1, or OATP1B3 up to 1000 μM . However, imeglimin inhibited uptake of typical substrates into cells expressing human OCT1 and OCT2, with K_i values of 154 and 146 μM , respectively; imeglimin also inhibited uptake of a typical substrate for MATE1 into MATE1-expressing cells, with an IC_{50} of 19.24 μM . It is unlikely that imeglimin inhibits OCT1 in clinical settings because its K_i value is about 21-fold greater than the clinical concentration observed of 7.5 μM . Similarly, it is unlikely that imeglimin inhibits OCT2 in vivo since the $I_{\text{max,u}}/\text{IC}_{50}$ value is <0.1 (CDER, 2020b), where $I_{\text{max,u}}$ is the maximal unbound plasma concentration of the interacting drug at steady-state. However, imeglimin has the potential to inhibit MATE1 ($I_{\text{max,u}}/\text{IC}_{50} > 0.1$). Therefore, a clinical DDI study with the recommended probe substrate (metformin) for MATE1 transporters was performed to assess the in vivo inhibition potential of imeglimin. Imeglimin coadministration had no effect on metformin systemic exposure. Metformin $\text{AUC}_{\text{over a dosing interval}}$ and C_{max} were 14% and 10% lower, respectively, when coadministered with

Fig. 10. Excretion balance after oral and intravenous administration of a single dose of 5 mg/kg of [14 C]imeglimin in rats (A) and dogs (B) and after oral administration of a single dose of 1000 mg [14 C]imeglimin in humans (C), PO: per os.

imeglimin. This fully demonstrated the lack of clinical inhibition of MATE1-mediated transport by imeglimin (Fouqueray et al., 2020).

In conclusion, these studies provide key information regarding the disposition characteristics of imeglimin, a novel oral antidiabetic drug targeting mitochondrial bioenergetics, in preclinical species and humans.

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Authorship Contributions

Participated in research design: Pascale, Sébastien.
Conducted experiments: Pascale, Sébastien.
Contributed new reagents or analytic tools: Sébastien.
Performed data analysis: Sébastien, Clémence.
Wrote or contributed to the writing of the manuscript: Clémence, Pascale, Sébastien.

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