Absorption, Distribution, Metabolism, and Excretion of [14C]iptacopan in Healthy Male Volunteers and in In Vivo and In Vitro Studies

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ABSTRACT

Iptacopan (LNP023) is an oral, small-molecule, first-in-class, highly potent proximal complement inhibitor that specifically binds factor B and inhibits the alternative complement pathway. Iptacopan is currently in development as a targeted treatment of paroxysmal nocturnal hemoglobinuria and multiple other complement-mediated diseases. In this study, the absorption, distribution, metabolism, and excretion (ADME) of iptacopan was characterized in six healthy volunteers after a single 100 mg oral dose of [14C]iptacopan. This was supplemented with an in vivo rat ADME study and metabolite exposure comparisons between human, rat, and dog, in addition to in vitro assays, to better understand the clearance pathways and enzymes involved in the metabolism of iptacopan. The fraction of [14C]iptacopan absorbed was estimated to be about 71%, with a time to maximum concentration of 1.5 hours and elimination half-life from plasma of 12.3 hours. Following a single dose of [14C]iptacopan, 71.5% of the radioactivity was recovered in feces and 24.8% in urine. [14C]iptacopan was primarily eliminated by hepatic metabolism. The main biotransformation pathways were oxidative metabolism via CYP2C8, with M2 being the major oxidative metabolite, and acyl glucuronidation via UGT1A1. The two acyl glucuronide metabolites in human plasma, M8 and M9, each accounted for ≤ 10% of the total circulating drug-related material; systemic exposure was also observed in toxicology studies in rat and dog, suggesting a low risk associated with these metabolites. Binding of iptacopan to its target, factor B, in the bloodstream led to a concentration-dependent blood:plasma distribution and plasma protein binding of [14C]iptacopan.

SIGNIFICANCE STATEMENT

We characterized the pharmacokinetics, excretion, metabolism and elimination of [14C]iptacopan (an oral, selective small-molecule inhibitor of factor B) in healthy human subjects. [14C]iptacopan was primarily eliminated by metabolism. The primary biotransformation pathways were oxidative metabolism via CYP2C8 and acyl glucuronidation via UGT1A1. Direct secretion of iptacopan into urine and potentially bile represented additional elimination mechanisms. Binding of iptacopan to its target, factor B, in the bloodstream led to a concentration-dependent blood:plasma distribution and plasma protein binding of [14C]iptacopan.

Introduction

The complement system, which comprises the classic, lectin, and alternative pathways, is central to the human innate immune response. It modulates adaptive immunity and is an important line of defense against pathogens. However, dysregulation of the alternative complement pathway, which also acts as an amplification loop of the classic and lectin pathways, either due to mutations in genes encoding complement or regulatory proteins or due to acquired factors, such as autoantibodies or nephritic factors, can lead to excessive complement activation and tissue damage, leading to morbidity (Merle et al., 2015; Morgan and Harris, 2015).

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ABBREVIATIONS: AAG, α1-acid glycoprotein; ADME, absorption, distribution, metabolism, and excretion; AE, adverse event; AUC, area under the concentration–time curve; AUC0–48h, area under the concentration–time curve from time 0 to 48 hours; AUCinf, the area under the concentration–time curve from time 0 extrapolated to infinity; AUClast, the area under the concentration–time curve from time 0 to time of last quantifiable concentration; C3G, complement 3 glomerulopathy; CL, systemic clearance; CL/F, the apparent oral clearance of the drug from the plasma; CYP, cytochrome P450 enzyme; EMA, European Medicines Agency; F, bioavailability; fb, fraction bound; FDA, Food and Drug Administration; fu, fraction unbound; HDL, high density lipoprotein; HLM, human liver microsomes; HPLC, high-performance liquid chromatography; HPLC-MS, high-performance liquid chromatography with mass spectrometry; HSA, human serum albumin; IgAN, immunoglobulin A nephropathy; Km, substrate concentration that yields a half-maximal velocity; KO, knockout; LC-MS, liquid chromatography with mass spectrometry; LDL, low-density lipoprotein; LSC, liquid scintillation counting; PK, pharmacokinetics; PNH, paroxysmal nocturnal hemoglobinuria; T1/2, elimination half-life; TEAE, treatment-emergent AE; Tmax, time to reach maximum radioactivity; UGT, uridine diphosphate glucuronosyltransferase; UPLC, ultra-performance liquid chromatography; VLDL, very low density lipoprotein; Vmax, maximum reaction velocity; Vz/F, apparent volume of distribution during the terminal phase; WT, wildtype.
Dysregulation of the complement system is implicated in the pathology of many rare diseases, including immunoglobulin A nephropathy (IgAN), complement 3 glomerulopathy (C3G), atypical hemolytic uremic syndrome, lupus nephritis, and paroxysmal nocturnal hemoglobinuria (PNH) (Morgan and Harris, 2015); apart from PNH, these are all kidney diseases. Both the structural and functional aspects of the kidney make them uniquely susceptible to complement-mediated damage (Poppelaars and Thurman, 2020).

Iptacopan (LNP023) is an oral, small-molecule, first-in-class, highly potent proximal complement inhibitor that specifically binds factor B and inhibits the alternative complement pathway (Schubart et al., 2019; Mainolfi et al., 2020). Iptacopan binds to the active site of factor B and its catalytically active fragment Bb (Schubart et al., 2019). Selective inhibition of factor B suppresses the activity of alternative pathway-related C3 convertase and the subsequent formation of C5 convertase (Schubart et al., 2019; Mainolfi et al., 2020). While iptacopan inhibits amplification of the classical and lectin pathways, it leaves direct signaling intact (Schubart et al., 2019). Currently in development as a targeted treatment of complement-mediated kidney diseases (IgAN, C3G, atypical hemolytic uremic syndrome, and lupus nephropathy) and PNH, iptacopan has a favorable safety profile and has been well tolerated in phase 1 and 2 clinical trials (Webb et al., 2020; Risitano et al., 2021; Wong et al., 2021a,b; Barratt et al., 2022; Jang et al., 2022).

Iptacopan was rapidly absorbed in healthy volunteers with no evidence of food effect and moderate plasma clearance (Webb et al., 2020). In healthy volunteers and patients, an underproportional dose-exposure relationship was reported, with a terminal half-life of approximately 20 hours (Webb et al., 2020; Risitano et al., 2021). The metabolic characteristics of iptacopan have not been previously reported.

This study was designed to evaluate the absorption, distribution, metabolism, and excretion (ADME) properties of iptacopan in healthy volunteers, as recommended by health authorities including the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), among others. The clinical study was supplemented with an in vivo rat ADME study and metabolite exposure comparisons between human, rat, and dog, as well as in vitro assays, to better elucidate the clearance pathways and enzymes involved in the metabolism of iptacopan to understand potential drug interactions and to characterize plasma protein binding.

Materials and Methods

Human ADME Study

Radiolabeled Study Drug. The parent batch of [14C]iptacopan was manufactured by Almac Sciences Ltd, UK, under Good Manufacturing Practice and released for human use according to predefined specifications. [14C]iptacopan capsules, each at a dose of 100 mg [14C]iptacopan (free base) with a specific activity of 3.7 MBq (100 µCi; 37 kBq/mg), were manufactured by PRA Health Sciences under Good Manufacturing Practice. Analyses of chemical and radiochemical identity, purity, stability, and release for human use was performed according to predefined and quality assurance-approved specifications. Radiochemical purity was 98.6%. The chemical structure of the compound and the position of the radiolabel are shown in Fig. 1. The estimated effective radiation burden after a single oral radiolabeled dose of 3.7 MBq [14C]iptacopan was 0.07 mSv; below the 0.1 mSv threshold considered to constitute a trivial risk and be acceptable for biologic investigations, based on available pharmacokinetic and rat tissue distribution data.

Study Design and Subjects. This was a single-center, open-label study to evaluate the ADME and pharmacokinetics (PK) of a single oral dose of [14C]iptacopan in healthy subjects conducted between June 24 and July 26, 2019. The study was performed by PRA Health Sciences and conducted according to International Council for Harmonization E6 Guidelines for Good Clinical Practice that have their origin in the Declaration of Helsinki. Informed consent was obtained in writing from each subject at screening before any assessments were performed.

Subjects were healthy males, ages 18–55 years, weighing 180 kg, who could communicate well with the investigator and understand and comply with the requirements of the study. Subjects were nonsmokers with no history of alcoholism or drug abuse, no use of any prescription drugs or herbal medication within the 4 weeks before dosing, and no intake of xanthine-containing food/beverages (e.g., caffeine) within 48 hours of study dosing. Subjects were excluded if they had any surgical or medical condition that might significantly alter the ADME of drugs or that could jeopardize subjects’ participation in the study or history or current evidence of active or latent tuberculosis, hepatitis B, hepatitis C, or human immunodeficiency virus. The full list of inclusion and exclusion criteria are provided in the Supplemental Materials. Subjects were expected to comply with dietary and fluid restrictions and undergo multiple blood draws and medical visits, as stipulated in the protocol.

The study consisted of a screening period of up to 28 days, a baseline period before drug administration (day –1), and a confinement period of 9 days after administration of a single dose of [14C]iptacopan on day 1. Eligible subjects remained domiciled from baseline (day –1) until sample collection for a period of up to 216 hours (9 days) post-dose (days 1–10). On day 1, following an overnight fast of at least 10 hours, subjects received a single, oral 100 mg dose of [14C]iptacopan; fasting continued for 1 hour after dosing. Following the day 1 dose, plasma, whole blood PK samples, and excreta were collected at the predefined timepoints listed later. On day 10, subjects left the study site and were contacted as soon as possible to notify them whether total radioactivity concentrations in their samples allowed them to be released from the study (>90% radioactivity recovery in excreta or the combined urinary and fecal excretion of <1% of the administered dose for 2 consecutive days and total radioactivity in plasma of <5% of the Cmax).

Study Objectives. The primary objectives were to characterize the plasma PK of iptacopan and its key metabolites, determine the PK of total radioactivity in blood and plasma, and determine the rates and routes of excretion of [14C]iptacopan-related radioactivity, including mass balance of total drug-related radioactivity in urine and feces following a single 100 mg oral dose of [14C]iptacopan. The secondary objective was to assess the safety and tolerability of a single 100 mg oral dose of [14C]iptacopan. Exploratory objectives included characterizing the plasma PK of iptacopan and its key metabolite(s), based on radiometry data, and identifying and semi-quantifying iptacopan and its metabolites in plasma and excreta (urine and feces) to elucidate key biotransformation pathways and clearance mechanisms of iptacopan in humans.

Safety Assessments. Adverse events (AEs) were monitored continuously throughout the study and consisted of monitoring and recording all AEs and regular monitoring of clinical laboratory measures, physical condition, vital signs, and electrocardiograms.

Sample Collection. Blood samples for PK, radioactivity, and metabolite identification were collected at screening, baseline (day –1), pre-dose on day 1, and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours post-dosing, and then every 24 hours until subjects were released from the study. Blood was taken by either direct venipuncture or by an indwelling cannula inserted in a forearm vein into K2-EDTA vacuum tubes for analysis of iptacopan in plasma and of total radioactivity in plasma and whole blood (nonacidified). In addition, blood was taken into Greiner Vacuette FC Mix tubes containing buffered Na2EDTA, sodium fluoride, citric acid, and sodium citrate (acidified) to prevent potential instability of expected acyl glucuronide metabolites (which could complicate metabolite profiling) and to investigate the potential impact of sample acidification on pharmacokinetic parameters. The plasma, acidified plasma, and blood samples intended for metabolism investigations were frozen immediately after sampling and were stored at –80°C until analysis. Urine samples for total radioactivity measurements and determination of metabolites were collected at baseline and at 0–6 hours, 6–12 hours, and 12–24 hours for the first 24 hours, and then at 24-hour intervals. Urine samples were stored at 4–8°C during the sampling periods. Thereafter, acidified and nonacidified aliquots were taken for various analyses. The acidified aliquots intended for metabolism investigations were frozen and stored at –80°C until analysis. For acidified urine samples, the pH was adjusted to 5.0 by the dropwise addition of a 70% lactic acid solution.
Feces samples for total radioactivity measurements and determination of metabolites were collected at baseline, 0–24 hours post-dosing, and then at 24-hour intervals. Feces homogenate aliquots intended for metabolism investigations were frozen and stored at –80°C until analysis.

**Pharmacokinetic Evaluations.** Iptacopan concentrations in non-acidified and acidified plasma were analyzed by Veeda Clinical Research Pvt. Ltd. (Gujarat, India) using a validated liquid chromatography tandem mass spectrometry method; the lower limit of quantification was 1 ng/mL. PK parameters were evaluated for iptacopan in plasma and for total radioactivity in plasma and whole blood. Cmax, time to reach maximum radioactivity (Tmax), the elimination half-life (T1/2), the area under the concentration–time curve from time 0 to the time of last quantifiable concentration (AUClast), the area under the concentration–time curve from time 0 extrapolated to infinity (AUCinf), the apparent oral clearance of the drug from the plasma (CL/F, for iptacopan only), the apparent volume of distribution (Vz/F, for iptacopan only). The PK of iptacopan (plasma) and total radioactivity (plasma and whole blood) were calculated based on concentration–time data for iptacopan (in ng/mL) and total radioactivity (in ng-eq/mL), respectively. PK were calculated using Phoenix WinNonlin 8.1 (Certara, Princeton, NJ, USA), with noncompartmental analysis.

**Total Radioactivity Measurement.** Total radioactivity concentrations in non-acidified whole blood and plasma, urine, and feces were analyzed by the PRA Bioanalytical Laboratory (Assen, The Netherlands) using a validated liquid scintillation counting (LSC) method with a typical counting time of 10 minutes. Blood samples (duplicates of each 300 µL blood, weighed) were measured after solubilization. Plasma (duplicates of each 250 µL plasma, weighed) was mixed directly with the liquid scintillation cocktail before radiometry. Feces samples (quadruplicates of approximately 0.5 g each, weighed) were measured after combustion. Urine samples (duplicates of 1 mL each) were measured directly with the cocktail. LSC was performed using a LSC model Tri-Carb 2200CA or Tri-Carb 3170TR/SL (Packard Instruments, Meriden, CT, USA) using an external standard ratio method for quench correction. The lower limit of quantification for plasma, blood, urine, and feces were 30, 40, 10, and 50 dpm/g, respectively, corresponding to 13.5, 18.0, 4.5, and 22.5 ng-eq/mL.

**Mass Balance.** Urinary and fecal recovery [in mg equivalent (eq)] and total recovery (mass balance) of radioactivity was derived. Cumulative amounts excreted in urine, feces, and total radioactivity were determined. Feces homogenate aliquots intended for metabolism investigations were frozen and stored at –80°C until analysis.

**Metabolite Profiling in Plasma and Excreta.** Metabolite profiling in plasma (up to 48 hours), urine, and feces (up to 96 hours) was performed by Novartis’ biotransformation group (Basel, Switzerland). Samples were pooled across all subjects to give single representative samples. Profiles of radioactive parent and metabolites in plasma, including key parameters [e.g., area under the concentration–time curve from time 0 to 48 h (AUC0–48), AUCinf, and T1/2] where possible, were performed in acidified plasma (1) to prevent acyl migration of acyl glucuronide metabolites (Patel, 2020) and (2) because there was no significant difference in the PK of iptacopan obtained in acidified and non-acidified samples.

Acidified plasma pools across subjects at 0.75, 1.5, 2, 2.5, 3, 6, 12, 24, and 48 hours post-dose were prepared by combining equal volumes of plasma from each subject for each timepoint. Pools were extracted as described in the Supplemental Materials. Total recovery of plasma extractions after sample preparation ranged from 89.2% to 100% for all timepoints up to 48 hours. For the 24- and 48-hour timepoints, total recovery after sample preparation was 100%. High performance liquid chromatography (HPLC) column recovery, carried out on a representative AUC pooled sample, was complete (100%). An acidified urine pool (0–96 hours, six subjects) was prepared by combining identical percentages of volumes of the individual urine fractions of all six subjects from 0 to 96 hours, representing 98.3% of the radioactivity excreted in urine. The urine pools were stored at ≤ –80°C until high performance liquid chromatography-mass spectrometry (HPLC-MS) analysis with offline radioactivity detection. A volume of 500 µL from the urine pool was directly injected into the HPLC-MS system with of-line radioactivity detection. HPLC column recovery was complete (100%). Individual subject feces pools (six subjects) from 0 to 96 hours were prepared by combining identical percentages of the individual feces homogenate weights collected from 0 to 96 hours. An average pool across the six subjects was prepared from the individual subject feces pools in the same way. Pools were extracted as described in the Supplemental Materials. The total recovery of the feces extraction after sample preparation was 90.6%. HPLC column recovery was complete (100%).

The structural characterization of metabolites in plasma and excreta was carried out by HPLC-MS/MS analysis, which provided protonated molecular ions and the key fragments used for structure assignment. Metabolite profiling of radioactive parent and metabolites in urine and feces included dose-proportion (%)...
per metabolite. The underlying biotransformation processes and the essential clearance mechanisms were derived as feasible. Chromatography was performed on an Agilent 1260 system binary pump equipped with a sample manager, an UV-visible spectrophotometer detector VWD, and a column manager. The software used was Agilent OpenLab CDS ChemStation A.02.09[017]. The components were separated at 50°C on a Waters HPLC symmetry C18 column (3.5 μm, 100 Å, 4.6 x 150 mm) protected by a Phenomenex security guard cartridge C18 (4 x 30 mm). Volumes of up to 500 μL were injected. A gradient HPLC method was used as described in the Supplemental Materials. After chromatography, the effluent was split in a ratio of approximately 20:80. The smaller amount (20%) was directed into the electrospray HPLC-MS interface and the larger part (80%) was used for UV detection followed by offline radioactivity detection.

Offline radioactivity detection allowed for peak correlation with mass spectral data. The effluent was collected in 6-second fractions into 96-well Lumaplates (Perkin Elmer). Fractions were evaporated to dryness, and the radioactivity was counted in a Microplate scintillation counter model Topcount NXT (Packard).

Offline radiochromatograms were evaluated using an in-house customized program NITISC based on Microsoft Excel software. The structures of metabolites were defined from their product ion spectra, the elemental composition by exact mass measurement, and hydrogen/deuterium exchange experiments and was supported by comparison with synthetic reference compounds where possible (compounds are referenced following the metabolite within the Results section).

Structural information on metabolites was obtained by HPLC-MS/MS using a time-of-flight mass spectrometer model Synapt G2 operated under MassLynx, Version 4.1 (Waters Corporation, Manchester, UK).

**Determination of Fraction of Radioactivity in Plasma.** The fraction of radioactivity in plasma was calculated according to the formula:

\[
F_p(\%) = \frac{C_p}{C_0} \times (1 - H) \times 100
\]

where \(F_p(\%)\) is the fraction of total radioactivity in plasma, \(C_p\) is the concentration of radioactivity in plasma, \(C_0\) is the concentration of radioactive in whole blood, and \(H\) is hematocrit (mean of two measurements taken at baseline and on day 3).

**In Vitro Investigations**

**Enzyme Phenotyping.** In vitro experiments were conducted in human liver microsomes (HLMs), as well as with recombinant human uridine diphosphate glucuronosyltransferase (UGT) and human cytochrome P450 (CYP) enzymes to characterize the enzymes involved in the main metabolic pathways of iptacopan.

Stock solutions of 100 mM \(^{14}\text{C}\)iptacopan were prepared in DMSO. Solutions were diluted appropriately with 100 mM potassium phosphate (pH 7.4) containing 5 mM MgCl₂ for incubations. Incubation of \(^{14}\text{C}\)iptacopan with these preparations was performed using an Agilent 1200 liquid chromatograph. Components were separated on a Poroshell 120, Phe-Hex column (particle size 2.7 μm, 4.6x150 mm, Agilent, CA, USA) protected by a 4.6 x 5 mm guard column of the same stationary phase and thermostat at 40°C. Elution was performed at a flow rate of 1.0 mL/min (HPLC conditions are provided in the Supplemental Materials). For online radioactivity detection, the effluent was mixed with a Flo-Scint A scintillation mixture at a flow rate of 1.0 mL/min. Radioactivity was detected using an HPLC radioactivity monitor equipped with a 0.2 mL flow cell (Berthold Technologies GmbH, Regensdorf, Switzerland).

Enzyme kinetic parameters, \(V_{\text{max}}\) and \(K_{\text{m}}\), for the biotransformation by HLMs and major metabolizing enzymes were calculated using SigmaPlot 12.1 (Enzyme Kinetics module version 1.1; SPSS Science Inc., Chicago, IL, USA). \(V_{\text{max}}\) and \(K_{\text{m}}\) were determined using the Michaelis-Menten model. Intrinsic clearance was calculated as \(V_{\text{max}}/K_{\text{m}}\). The calculated unbound intrinsic clearance values and hepatic abundance information were used to obtain the fractions metabolized by individual enzymes (\(\text{fm, CYP2C8 and fm, CYP2D6}\)) to oxidative metabolism in liver.

**Blood Distribution and Plasma Protein Binding.** Samples for blood distribution and plasma protein binding analyses were obtained from healthy rats, dogs, humans, and wildtype (WT) and factor B knockout (KO) mice, and reagents were prepared as noted in the Supplemental Materials.

The in vitro blood/plasma distribution was investigated at nominal concentrations of 10, 100, 1000, and 10,000 ng/mL. \(^{14}\text{C}\)iptacopan was calculated based on the determination of total radioactivity using the specific radioactivity (5.015 MBq/mg free base). Whole blood hematocrit was determined in triplicate after centrifugation in micro-hematocrit capillaries (13000 g, 5 minutes). The fraction of compound in plasma (\(f_p\)) was calculated as follows:

\[ f_p(%) = \frac{C_p/C_b}{(1-H)} \times 100 \]

where \(f_p(%)\) is the fraction of total radioactivity in plasma, \(C_p\) is the concentration of radioactivity in plasma, \(C_b\) is the concentration in the buffer (100 g/mL), and \(H\) is the hematocrit value.

Equilibrium dialysis was used to analyze plasma protein binding in whole plasma and protein solutions containing either human serum albumin (HSA), α1-acid glycoprotein (AAG), or the lipoproteins high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). Stock solutions of \(^{14}\text{C}\)iptacopan were spiked (1:200, v/v) into plasma and protein solutions to obtain intended final concentrations of 10 (only plasma), 100, 1000, and 10,000 ng/mL (final ethanol concentration: ≤ 0.5%). Aliquots of 0.2 mL of spiked plasma/protein solutions were dialyzed (without precipitation) against phosphate-buffered saline for 6 hours at 37°C (n = 1–4 for each concentration/species). \(^{14}\text{C}\)iptacopan concentration was determined after dialysis in the buffer compartment (Cu) and the plasma/protein solution compartment (Cp) of each incubation. The unbound fraction in plasma (\(f_u\)) was calculated as follows:

\[ f_u(%) = \frac{C_u/C_p \times 100}{C_u/C_p \times 100} \]

where \(f_u(%)\) is the fraction of total radioactivity in plasma, \(C_u\) is the concentration of radioactivity in the buffer compartment (Cu), and \(C_p\) is the concentration of radioactivity in plasma.

Radioactivity was measured by LSC using a Packard Tri carb liquid scintillation counter. Concentrations of radiolabeled substances in blood distribution experiments were determined in weighed samples. Data were converted from Bq/g to ng/mL, assuming a density of 1.00 g/mL for all samples and using the specific radioactivity of the test compound.

**Other In Vitro Methods.** Descriptions of all other in vitro methods are provided in the Supplemental Materials.

**Results**

**Subject Demographics, Disposition, and Safety**

Six male subjects ages 21–46 years with a body mass index of 22.5–28.3 kg/m² were enrolled and received the study drug. None of the subjects withdrew or dropped out during the study. Five subjects were Caucasian, and one was Native American.

A single oral dose of 100 mg \(^{14}\text{C}\)iptacopan was well tolerated in healthy male subjects. No clinically relevant changes were observed in clinical laboratory assessments, vital signs, electrocardiograms parameters, and physical examinations. In total, eight treatment-emergent adverse events (TEAEs) regardless of study drug relationship were reported in 3/6 subjects (50.0%). Two subjects (33.3%) experienced headache, and one subject each experienced eyelid irritation, abdominal discomfort, fatigue (reported twice by one subject), oral herpes, and headache, experienced by one subject each. All TEAEs were mild and resolved by the end of the study. No deaths or serious AEs were reported. There were no study discontinuations due to TEAEs.
PK of Radioactivity and Iptacopan

After a single oral administration of a capsule containing 100 mg and 3.7 MBq (100 μCi) of [14C]iptacopan, iptacopan was rapidly absorbed with a median $T_{\text{max}}$ of 1.51 hours. Quantifiable concentrations of iptacopan in both non-acidiﬁed and acidiﬁed plasma were observed 15 minutes following dosing (the ﬁrst sampling timepoint) for all subjects. Iptacopan concentrations were quantifiable in plasma up to 96 hours post-dose in all six subjects, up to 120 hours post-dose in four subjects, and up to 168 hours post-dose in one subject.

Arithmetic mean iptacopan concentration–time proﬁles and PK parameters for non-acidiﬁed and acidiﬁed plasma were similar (Fig. 2A, Table 1). Iptacopan exposure (AUC$\text{inf}$) in plasma was 83.2% of the plasma total radioactivity AUC$\text{inf}$, indicating limited plasma exposure to metabolites. Blood total radioactivity AUC$\text{inf}$ was 66.2% of plasma total radioactivity AUC$\text{inf}$ (Fig. 2B, Table 1), indicating preferential distribution of iptacopan and metabolites toward plasma rather than blood.

The PK of iptacopan was characterized by medium to low clearance with a mean CL/F of 4.35 L/h and a mean apparent $T_{1/2}$ of 12.3 hours (Table 1). Interindividual variability of PK of iptacopan in plasma and total radioactivity in plasma and whole blood were low.

Iptacopan Blood Distribution and Plasma Protein Binding

In healthy human subjects, the fraction of total radioactivity in plasma decreased with increasing total radioactivity concentration in plasma (Fig. 3A). In vitro blood distribution and plasma protein binding (concentration dependency) of [14C]iptacopan were investigated for rat, dog, and human blood. Within the investigated concentration range (10–10,000 ng/mL), [14C]iptacopan plasma protein binding was concentration dependent for all species investigated. At the lowest concentration of 10 ng/mL, [14C]iptacopan was highly bound to plasma proteins, but this decreased in a concentration-dependent manner with increasing concentrations of [14C]iptacopan (Fig. 3B).

To investigate potential plasma protein binding of [14C]iptacopan to its target complement factor B, plasma protein binding parameters were evaluated in plasma from WT and factor B KO mice. [14C]iptacopan plasma protein binding was concentration dependent in WT mice...
but not for factor B KO mice. The mean fu at the lowest concentration studied in KO mouse was similar to the mean fu at the highest concentration studied in WT mouse (Fig. 3B), suggesting that the observed concentration-dependent plasma protein binding in WT mouse was due to saturation of binding to the complement factor B protein.

Finally, the concentration dependency of in vitro protein binding of [14C]iptacopan was assessed for isolated human plasma proteins: HSA, AAG, and a mixture of lipoproteins (HDL, LDL, and VLDL). [14C]Iptacopan was weakly bound to all investigated isolated plasma proteins (Fig. 3B), and binding was not concentration dependent, in contrast to results obtained with complete human plasma protein fractions. The mean (±SD) calculated fu for HSA, AAG, and lipoproteins over the range of 100–10,000 ng/mL [14C]iptacopan were 71.6% (± 3.3), 90.7% (± 0.4), and 84.0% (± 0.9), respectively. Taken together, these results indicate that iptacopan predominantly binds to plasma factor B and HSA.

Excretion of Radiolabeled Components
Mean recovery of total radioactivity was 96.4% of the administered dose (Table 2). Radioactivity was excreted predominantly in feces (on average 71.5% in feces versus 24.8% in urine). On average, 24.7% of radioactivity was recovered in urine within 72 hours, with 71.1% of the radioactivity being recovered in feces within 120 hours post-dose. By 216 hours post-dose, the excretion of radioactivity was complete (mean 96.4%), with low interindividual variability (range: 94.0%–98.5%; Fig. 4).

Metabolite Patterns in Plasma, Urine, and Feces
Metabolite profiles in acidified plasma were investigated up to 48 hours following iptacopan administration. Iptacopan was the most abundant compound in acidified plasma, representing 83% of the plasma [14C]AUC0–48h (Table 3, Fig. 5), in agreement with observations from pharmacokinetic profiling in plasma (Table 1). Of the two metabolites detected in acidified plasma, M8 (MHT297—direct acyl glucuronide of iptacopan) was the most abundant, accounting for 8.1% of the plasma [14C]AUC0–48h. A second acyl glucuronide, M9 [OHJ739—acyl glucuronide of metabolite M2 (JKHS04)] was present in lower

### TABLE 1
Summary of iptacopan and total radioactivity PK derived following a single oral dose of 100 mg [14C]iptacopan to six healthy male volunteers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nonacidified Plasma</th>
<th>Acidified Plasma</th>
<th>Plasma</th>
<th>Total Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_max, h</td>
<td>24.8 (1.71)</td>
<td>21.5 (1.65)</td>
<td>20.8</td>
<td>19.8 (1.64)</td>
</tr>
<tr>
<td>C_max, ng/mL or ng-eq/mL</td>
<td>24.8 (1.71)</td>
<td>21.5 (1.65)</td>
<td>20.8</td>
<td>19.8 (1.64)</td>
</tr>
<tr>
<td>AUC0–48h, ng/mL or ng-eq/mL</td>
<td>22,983 (18.9)</td>
<td>24,264 (20.6)</td>
<td>27,062 (12.1)</td>
<td>24,264 (20.6)</td>
</tr>
<tr>
<td>% of [14C]AUC0–48h</td>
<td>83.2 (12.2)</td>
<td>87.8 (14.1)</td>
<td>100 [reference]</td>
<td>66.2 (6.8)</td>
</tr>
<tr>
<td>% of [14C]AUClast</td>
<td>84.8 (12.3)</td>
<td>89.5 (14.0)</td>
<td>100 [reference]</td>
<td>63.2 (8.7)</td>
</tr>
<tr>
<td>Fp (%) as a function of total radioactivity concentration (ng-eq/mL) in plasma</td>
<td>140</td>
<td>120</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>y = 0.0145x + 100.61</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>R²</td>
<td>0.5195</td>
<td>0.5195</td>
<td>0.5195</td>
<td>0.5195</td>
</tr>
</tbody>
</table>

Values are geo-mean [CV (%)] except for Tmax, which is median (range).
Units are ng/mL for iptacopan plasma and ng-eq/mL for total radioactivity.

Fig. 3. (A) Fraction of total radioactivity located in the plasma compartment in healthy volunteers and (B) in vitro protein binding of [14C]iptacopan to rat, dog, human, and WT and factor B KO mice plasma and to isolated human plasma proteins. (B) Blood spiked with different concentrations of [14C]iptacopan was incubated for 0.5 h at 37°C. Fraction unbound of [14C]iptacopan in plasma of rat, dog, human, and WT and KO mice and after incubation with isolated human plasma proteins (HSA, AAG, and lipoproteins). The total radioactivity in plasma and dialysate was determined in triplicate (exception: triplicate for WT mouse with iptacopan 10–100 ng/mL, KO mouse, and rat; duplicate for human plasma with iptacopan 10 ng/mL). Values are given as mean ± SD (except for human plasma with iptacopan concentration of 10 ng/mL: mean of n = 2 only).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fe (%) urine</th>
<th>Fe (%) feces</th>
<th>Fe (%) total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>24.8 (1.71)</td>
<td>71.5 (2.79)</td>
<td>96.4 (1.63)</td>
</tr>
<tr>
<td>CV (%) mean</td>
<td>6.9</td>
<td>3.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Median (range)</td>
<td>25.2 (21.9, 27.1)</td>
<td>72.0 (66.9, 74.2)</td>
<td>96.7 (94.0, 98.5)</td>
</tr>
</tbody>
</table>

CV% = (SD/mean)*100.
Fe, fraction excreted.
abundance (5.2% of plasma \(14\text{C-AUC}_{0-48h}\); Table 3, Fig. 5) Extrapolation to AUC_{inf} gave similar results, with iptacopan, M8, and M9 accounting for 87.9%, 7.30%, and 4.76% of the total \(14\text{C-AUC}_{inf}\), respectively. Overall, > 96% of the detected radioactive components of the plasma \(14\text{C-AUC}_{0-48h}\) could be assigned to the parent drug and structurally characterized metabolites.

A total of seven drug-related components were identified in acidified urine (Fig. 6A). The most abundant was iptacopan, which represented 17.9% of the administered dose. The most abundant metabolites were M1 (KVBO52, formed by N-dealkylation) and the acyl glucuronide M9 (OHJ739), which represented 3.8% and 1.6% of the dose, respectively. The other metabolites detected in urine [P3.1, M2 (JKH504), M6 (OH443), and M8 (MHT297)] were minor, representing \(\leq 0.5\%\) of the dose each (Table 4); > 98% of the total amount of radioactive components excreted in urine could be attributed to iptacopan and structurally characterized metabolites.

A total of seven drug-related components were identified in feces (Fig. 6B). The most abundant were metabolite M2 (JKH504), iptacopan, and metabolite M7, which represented 26.7%, 16.8%, and 8.3% of the dose, respectively. The other metabolites detected in feces [M11, M6 (ODH443), M8 (MHT297), M12, and M13] were minor, representing \(\leq 4.2\%\) of the dose each (Table 4); > 87% of the total amount of the radioactive components excreted in feces could be attributed to iptacopan and structurally characterized metabolites.

The metabolism of iptacopan in humans was mainly oxidative (Fig. 1), with direct oxidative metabolites (M1, M2, M6, M7, M11, M13) and secondary metabolites thereof (M9, M12) accounting for 51.8% of the administered dose (Table 4). Direct acyl glucuronidation is a smaller pathway. Assuming that the drug and metabolites are stable against intestinal bacterial enzymes, the mean oral absorption of iptacopan was estimated to be at least 70.6% of the administered dose (24.8% of urinary excreted radioactivity plus 45.8% of dose in feces attributable to metabolites). Based on the recovered amount of parent in feces (16.8%) and assuming biliary excretion and consequent cleavage of M8 by intestinal bacterial, the fraction absorbed is likely higher.

Excretion and Metabolite Patterns in Rat

After oral administration of \([14\text{C}]\)iptacopan 10 mg/kg to male rats, absorption was high (81.8%–85.8%) and bioavailability was 49.7%, indicating a moderate bioavailability and first-pass effect. As in the human study, radioactivity was excreted predominantly in the feces (94.4 ± 1.06% versus 5.09 ± 0.193% in the urine within 168 hours). In the rat, iptacopan was predominantly eliminated via metabolism (Fig. 1). In bile duct-cannulated rats, radioactivity was predominantly excreted in bile (78.9%).

Similar to humans, iptacopan was the major component (91.0% of \(14\text{C-AUC}_{last}\) in the plasma after oral administration. The metabolite M8 and additional metabolites (M1, M2, and M4) were detected at low levels (< 5% of \(14\text{C-AUC}_{last}\)). In urine, M1 was the major metabolite (2.17% of dose), as observed in humans. Other metabolites were detected in trace amounts (< 0.5% of dose; Supplementary Table 2). In feces, iptacopan was the major component after oral administration (36.1% of dose). As observed in humans, M2 was the major metabolite in feces (29.3% of dose). Metabolites M1,
M4, M6, and M7 were detected in lower amounts (< 10% of the dose; Supplementary Table 2).

In the bile of bile duct-cannulated rats, the major metabolites were M8 and isomers (20.4% of dose), M2 (20.2% of dose), and iptacopan (11.7% of dose; Supplementary Table 3). Only 3.9% of total radioactivity was excreted in the feces of bile duct-cannulated rats, 3.5% of which was iptacopan (Supplementary Table 3).

Coverage of Circulating Metabolites in Other Studies of Iptacopan

The most abundant circulating acyl glucuronide metabolites M8 and M9 identified in healthy human subjects in this study were also observed in the first-in-human phase 1 dose-finding study following multiple doses of oral iptacopan 200 mg twice daily and in toxicity studies in rats and dogs following multiple doses [750 mg/kg/d (rat) and 150 mg/kg/d (dog)].

Fig. 5. (A) PK of RA, iptacopan, and main metabolites in acidified plasma and (B) representative radiochromatogram of acidified plasma. Mean concentrations of iptacopan and its main metabolites in acidified plasma were semi-quantitatively determined from the integrated peaks of the radiochromatograms. Synthetic reference compounds are noted in parentheses. RA, radioactivity.

Fig. 6. Radiochromatograms of (A) urine and (B) feces from 6 healthy male subjects, pooled following a single oral dose of 100 mg [14C]iptacopan. Synthetic reference compounds are noted in parentheses.
Iptacopan Metabolism and Disposition in Healthy Volunteers

The total amount of metabolites (% of dose) excreted in pooled urine or feces of six subjects

<table>
<thead>
<tr>
<th>Component</th>
<th>Biotransformation</th>
<th>Urine</th>
<th>Feces</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3.1</td>
<td>Unknown</td>
<td>0.459</td>
<td>–</td>
<td>0.459</td>
</tr>
<tr>
<td>M11</td>
<td></td>
<td>2.49</td>
<td>2.49</td>
<td></td>
</tr>
<tr>
<td>M1 (KVB052)</td>
<td>O-deethylation, dioxgenation and de-hydrogenation</td>
<td>3.82</td>
<td>3.82</td>
<td></td>
</tr>
<tr>
<td>M9 (OHJ739)</td>
<td>O-deethylation, acyl glucuronidation</td>
<td>1.60</td>
<td>1.60</td>
<td></td>
</tr>
<tr>
<td>M2 (JKH504)</td>
<td>O-deethylation</td>
<td>0.268</td>
<td>26.7</td>
<td>27.0</td>
</tr>
<tr>
<td>M7</td>
<td>Di-oxidation and de-hydrogenation (carboxylation)</td>
<td>–</td>
<td>8.32</td>
<td>8.32</td>
</tr>
<tr>
<td>M6 (OHJ443)</td>
<td>Hydroxylation</td>
<td>0.359</td>
<td>2.01</td>
<td>2.37</td>
</tr>
<tr>
<td>M8 (MHT297)</td>
<td>Acyl glucuronidation</td>
<td>0.345</td>
<td>–</td>
<td>0.345</td>
</tr>
<tr>
<td>Iptacopan</td>
<td></td>
<td>17.9</td>
<td>16.8</td>
<td>34.8</td>
</tr>
<tr>
<td>M12</td>
<td>O-deethylation + conjugation with Unknown component</td>
<td>–</td>
<td>4.22</td>
<td>4.22</td>
</tr>
<tr>
<td>M13</td>
<td>Conjugation with unknown Component</td>
<td>–</td>
<td>2.03</td>
<td>2.00</td>
</tr>
<tr>
<td>Total detected</td>
<td></td>
<td>24.8</td>
<td>62.7</td>
<td>87.4</td>
</tr>
<tr>
<td>Lost during sample processing</td>
<td></td>
<td>–</td>
<td>6.50</td>
<td>6.50</td>
</tr>
<tr>
<td>Total analyzed (pool 0–96 h)</td>
<td></td>
<td>24.8</td>
<td>69.2</td>
<td>93.9</td>
</tr>
<tr>
<td>Total excretion (0–216 h)</td>
<td></td>
<td>24.8</td>
<td>71.5</td>
<td>96.4</td>
</tr>
</tbody>
</table>

Synthetic reference compounds are noted in parentheses. Data are derived from metabolite patterns and components are listed in order of elution. Dash indicates not detected.

*Combined losses due to sample extraction and reconstitution. No losses were observed in HPLC column recovery experiments.

kg/d (dog) of iptacopan. Exposure coverage analyses of circulating metabolites, conducted in the human and toxicity samples using the method described by Gao et al. (Gao et al., 2010), showed that M8 and isomers had rat:human and dog:human plasma ratios > 0.5 (range: 0.688–2.72), and M9 had rat:human and dog:human plasma ratios < 0.5 (range: 0.010–0.022).

In Vitro Acyl Glucuronide Stability Investigations

The in vitro half-life of the acyl glucuronide metabolites M9 (OHJ739) and M8 (MHT297), detected in human plasma and urine, was investigated in phosphate buffer and formation of the parent molecule iptacopan and potential acyl glucuronide isomers monitored.

The half-life of M9 in phosphate buffer was 2.0 hours. Liquid chromatography with mass spectroscopy analyses showed the formation of several acyl glucuronide isomers but no hydrolysis to parent compound during a 24-hour incubation.

The half-life of M8 in phosphate buffer was 1.6 hours. The stability of M8 in human blood, plasma and urine and rat urine were also assessed. M8 was stable at low pH (2.9–6.5) and temperature (4°C). Liquid chromatography with mass spectroscopy analyses showed the formation of acyl glucuronide isomers and hydrolysis to iptacopan.

In Vitro Metabolism of [14C]iptacopan in Human Liver Microsomes and by Recombinant Enzymes

The biotransformation of iptacopan in HLMs was slow, with an apparent intrinsic clearance of 0.02–1.27 mL/min/mg (Supplementary Table 4), in agreement with the PK observed in the human study. To identify the enzymes involved in the oxidative metabolism and direct acyl glucuronidation of iptacopan, a number of in vitro investigations were conducted. Incubation of [14C]iptacopan in HLMs in the presence of NADPH formed two oxidative metabolites: M2 (O-deethylation) and M6 (C-oxidation). M6 formation was observed by incubating iptacopan with recombiant human (rh) CYP1A1, CYP2C8, and CYP3D6 enzymes; CYP1A1 only formed M6 and no other metabolites. M2 formation was only catalyzed by rhCYP2C8. A weak turnover of < 2% was observed in the three recombinant enzymes. The Michaelis-Menten apparent unbound $K_m$ was 32 mM and $V_{max}$ was 2.6 pmol/min/mg protein for rhCYP2C8 (Supplementary Table 4). For rhCYP2D6, low intrinsic clearance was measured, and enzyme kinetics could not be performed due to this low turnover. Based on these results, estimated contributions of CYP2C8 and CYP2D6 to the oxidative metabolism of iptacopan were approximately 98% and 2%, respectively.

After incubation with [14C]iptacopan in HLM and in the presence of uridine diphosphate glucuronic acid, two direct glucuronide conjugates were formed: M8 (MHT297; direct O-glucuronide) and, to a lesser extent, M8c (isomerized M8, O-glucuronidation). M8 and M8c formation only correlated with UGT1A1 specific marker activity, with a Michaelis-Menten apparent unbound $K_m$ of 247 µM and $V_{max}$ of 296 pmol/min/mg protein (Supplementary Table 4). However, recombinant UGT mapping in HLMs also showed significant iptacopan turnover by UGT1A3 and UGT1A8. Based on these results, UGT1A1 was considered the major enzyme involved in direct glucuronidation of iptacopan in the liver; however, the involvement of other UGTs in the liver or in extra-hepatic tissues cannot be excluded.

**Discussion**

Overall, a single oral dose of 100 mg [14C]iptacopan was well tolerated in healthy male volunteers. Iptacopan was rapidly absorbed with a median $T_{max}$ of 1.51 hours. PK of total radioactivity were comparable with that of iptacopan, indicating that metabolites did not accumulate in plasma. PK of iptacopan were characterized by a medium to low clearance with a mean apparent $T_{1/2}$ of 12.3 hours.

Excretion of total radioactivity was complete by six days following study drug administration. Radioactivity after oral dosing in both humans and rats was excreted predominantly in feces. The mean oral absorption of iptacopan in humans was estimated to be at least 70.6% of the administered dose. Although bioavailability was not calculated in the human ADME study, bioavailability was calculated as approximately 50% in the rat ADME study following an 82%–86% absorption rate, indicating that a moderate intestinal/hepatic first-pass effect in human could be expected. Approximately 18% of iptacopan was detected in the urine of humans, suggesting a minor contribution of renal clearance to the total clearance. In addition, approximately 17% of iptacopan was found in feces, likely derived from a mix of nonabsorbed material, minor biliary excretion of the parent compound, and cleavage of M8 in...
the gastrointestinal tract. Therefore, metabolism is considered as the predominant route of elimination (approximately 53% of dose). Iptacopan is being investigated in PNH and multiple other complement-mediated diseases. As hepatic metabolism is the major route of elimination, the impact of kidney impairment in the clearance of iptacopan may be low.

The current data indicate that phase I metabolism is the major contributor to the clearance of iptacopan, with oxidative metabolites accounting for approximately 50% of the administered dose. M2, formed by O-deethylation, was the major metabolite identified in feces from both human and rat ADME studies (26.7% and 29.3% of the dose, respectively). Kinetic analyses suggest that CYP2C8 is the predominant contributor (98%) to the oxidative metabolism of iptacopan. The expression of CYP1A1 is highly variable (Lang et al., 2019); however, based on the low amounts of M6 in excreta (2.4%), no relevant contribution by CYP1A1 to hepatic or extra-hepatic metabolism of iptacopan is expected. CYP2C8 is abundant in human liver and is known to metabolize > 100 clinically used drugs (Backman et al., 2016). Given the predominant contribution of CYP2C8 in the metabolic elimination of iptacopan, a drug–drug interaction study with the CYP2C8 inhibitor clopidogrel has recently been conducted to provide guidance for the coadministration of iptacopan and CYP2C8 inhibitors, and a manuscript is in preparation.

Acyl glucuronidation was an additional minor metabolic elimination pathway. M8 and M9 were the only metabolites detected in human plasma. Overall, extrapolated AUC_{inf} of plasma metabolites were similar to AUC_{O-48h}. Suggesting that metabolites are eliminated rapidly and, assuming that AUC_{inf} is equivalent to AUC_{t-last}, show that significant accumulation on multiple dosing is unlikely. In rats, M8 and M9 were not detected in feces but were detected in bile, suggesting that they are hydrolyzed back to the parent drug and metabolite M2 by microflora in the gastrointestinal tract. In feces of bile duct-cannulated rats, little iptacopan was detected (3.5% of the dose), suggesting that minor gastrointestinal secretions occur. In vitro studies suggest that UGT1A1 is the key enzyme involved in hepatic glucuronidation of iptacopan in human liver. However, the involvement of other UGTs in the liver or extra-hepatic tissues, such as UGT1A3 and UGT1A8, cannot be excluded. Although we did not quantitatively assess the fraction of metabolites produced by CYP versus UGT enzymes, UGT1A1 is likely to have a minor contribution to the metabolism of iptacopan. Assuming that all unchanged iptacopan in feces (16.8%) resulted from hydrolysis of M8 upon biliary secretion, glucuronidation would maximally account for approximately 20% of iptacopan elimination. A similar contribution was also observed in bile duct-cannulated rats (approximately 20%).

Acyl glucuronides are phase II metabolites that can be unstable and highly reactive under physiologic conditions. They therefore pose a concern from a toxicological perspective and have been associated with idiosyncratic toxic reactions (Van Vleet et al., 2017). However, evidence suggests that acyl glucuronide formation alone does not pose an increased risk compared with other metabolites, and chemical reactivity assays, in vitro assessments, and evaluation of exposure in toxicology species are recommended in early clinical development to assess the risk-benefit of the parent drug (Walles et al., 2020). Importantly, in the current study, circulating acyl glucuronide metabolites M8 and M9 each accounted for < 10% of the total drug-related exposure and were therefore not clinically significant in relation to EMA and FDA safety testing guidance for drug metabolites (https://www.ema.europa.eu/en/ich-m3-r2-non-clinical-safety-studies-conduct-human-clinical-trials-pharmaceuticals; https://www.fda.gov/media/72279/download). In toxicity studies, exposure to the acyl glucuronide metabolite M8 at the no-observed-adverse-effect level was similar to or higher than human exposure at the highest phase 3 dose (200 mg twice daily). Exposure to the minor circulating metabolite M9 was lower but was detected in the circulation of both rats and dogs and in the bile of rats (approximately 3% of dose). M8 and M9 had a T_{1/2} in phosphate buffer (pH 7.4) of 1.6 and 2 hours, with some hydrolysis to parent compound observed for M8 but not M9. Overall, these data suggest a low risk associated with circulating acyl glucuronide metabolites of iptacopan.

Total radioactivity exposure with [14C]iptacopan was higher in plasma compared with whole blood, indicating preferential distribution of drug-related material to plasma. Analysis of human radioactivity in blood showed clear concentration dependency in the blood/plasma distribution of total radioactivity, in agreement with in vitro studies in human, rat, and dog blood. In vitro, the binding of iptacopan to isolated AAG and lipoproteins was not concentration dependent and only weakly bound to HSA, in contrast to complete human plasma protein fractions, indicating that iptacopan predominantly binds to plasma factor B and HSA. Studies in WT and factor B KO mice showed concentration dependency in WT but not KO mice, suggesting that concentration-dependent plasma protein binding in WT mice was primarily due to saturation of binding to complement factor B. The saturation of target binding at higher doses of iptacopan is linked to the pharmacodynamics and efficacy of iptacopan. Preclinically, iptacopan has demonstrated high-affinity binding to (K_D 0.0079±0.0019 μM) and potent inhibition of human factor B (IC_{50} 0.01±0.006 μM) (Schubart et al., 2019). In phase 2 clinical studies of IgAN and C3G, iptacopan demonstrated dose-dependent inhibition of alternative complement pathway biomarkers, including plasma Bb, serum Wieslab, and urinary C5b-9, with full inhibition reached with iptacopan 100–200 mg twice daily (Wong et al., 2021b; Barratt et al., 2022).

In conclusion, this study met all of its objectives. The recovery of 100 mg [14C]iptacopan was complete in the human ADME study, with 71.5% of the dose recovered in feces and 24.8% in urine. The fraction absorbed was estimated to be at least 70.6%. Elimination is mainly driven by oxidative metabolism, via CYP2C8. Direct acyl glucuronidation appeared to be a minor pathway, predominantly involving UGT1A1. In addition, direct renal and potentially biliary secretion contributed to the elimination of iptacopan. The two acyl glucuronide metabolites in human plasma, M8 and M9, comprised 7.5% and 4.8% of total circulating drug-related material, respectively, based on AUC_{t-last} and AUC_{inf}. M8 exposure at the highest phase 3 dose (200 mg twice daily) was covered by the toxicology species rat and dog. The metabolite M9 was also detected in rat and dog plasma at exposures lower than the human exposure, and M9 was also detected in rat bile, indicating that rat liver was exposed to the metabolite. Binding of iptacopan to its target, factor B, in the bloodstream led to a concentration-dependent blood–plasma distribution and plasma protein binding of [14C]iptacopan. These pharmacokinetic, excretion, metabolism, and elimination findings suggest the evaluation of iptacopan for appropriate clinical conditions is warranted.

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Data Availability

The authors declare that all the data supporting the findings of this study are available within the paper and its Supplemental Data.
Iptacopan Metabolism and Disposition in Healthy Volunteers

Authorship Contributions

Participated in research design: James, Poller, Klein, Pearson.
Conducted experiments: Romeo, Pearson.
Contributed new reagents or analytic tools: Romeo.
Performed data analysis: James, Poller, Romeo, Klein, Pearson.
Wrote or contributed to the writing of the manuscript: James, Kulmatycki, Poller, Romeo, Van Lier (PI of the clinical study), Klein, Pearson.

References


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