ORIGINAL RESEARCH ARTICLE

Pharmacokinetics and Drug–Drug Interaction of Ocedurenone (KBP-5074) in vitro and in vivo

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Abstract

Background and Objectives Ocedurenone (KBP-5074) is a novel nonsteroidal mineralocorticoid receptor antagonist that has demonstrated safety and efficacy in clinical trials in patients with uncontrolled hypertension and stage 3b/4 chronic kidney disease. This study evaluated the involvement of cytochrome P450 (CYP) isozymes and drug transporters in the biotransformation of ocedurenone, and whether ocedurenone inhibited or induced CYP enzymes and transporters. Clinical pharmacokinetic drug–drug interaction (DDI) of ocedurenone with CYP3A inhibitor and inducer were investigated in healthy volunteers.

Methods In vitro tests were conducted to determine which CYP enzymes were involved in ocedurenone's metabolism and whether ocedurenone inhibited or induced these CYP enzymes; ocedurenone substrate characteristics for efflux and uptake transporters and its inhibitory potential on major drug transporters were also assessed. A clinical DDI study was conducted in healthy volunteers to evaluate the effects of a strong CYP3A inhibitor (itraconazole) and inducer (rifampin) on ocedurenone's pharmacokinetics.

Results The in vitro study showed that ocedurenone was primarily metabolized by CYP3A4 and that it did not inhibit CYP enzymes. Ocedurenone appeared to be a substrate of BCRP and P-gp efflux transporters and inhibited BCRP, BSEP, MDR1, MATE1 and 2-K, OATP1B1/3, and OCT1. The clinical DDI study showed that itraconazole reduced ocedurenone's oral clearance by 51% and increased area under the plasma concentration–time curve extrapolated to infinity (AUC_{0-inf}) by 104%, while rifampin increased its oral clearance by 6.4-fold and decreased plasma AUC_{0-inf} by 84%.

Conclusion Ocedurenone was shown to be a CYP3A substrate, with no inhibition potential on major drug metabolizing CYP enzymes and transporters at clinical efficacious doses. Ocedurenone did not induce CYP1A2 and 3A4 activity in cultured human primary hepatocytes. Clinical DDI study indicated ocedurenone was well tolerated when administered as a single 0.5-mg dose both alone and with itraconazole or rifampin, and while itraconazole had a weak effect on ocedurenone's pharmacokinetics, rifampin had a significant effect reducing systemic exposures.

Key Points
Ocedurenone was a CYP3A substrate, it had no inhibition potential on major drug metabolism CYP enzymes and did not induce CYP1A2 and 3A4 activity in human hepatocytes.
Ocedurenone appeared to be a P-gp substrate and had inhibition potential on P-gp and BCRP.
Clinical pharmacokinetic DDI study indicated that co-administration of itraconazole resulted in a 104% increase in AUC_{0-inf}, while co-administration of rifampin resulted in an 84% reduction in AUC_{0-inf}.

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1 Introduction

Chronic kidney disease (CKD) is defined by kidney damage and/or an estimated glomerular filtration rate < 60 mL/min/1.73 m^2 for at least 3 month [1]. CKD incidence and prevalence are rising precipitously, with CKD now the third most rapidly increasing cause of death internationally [2, 3]. This increase can be attributed to a variety of factors, including an older population, poor adherence to treatment, low detection rates, and increasing rates of type 2 diabetes and hypertension [4–6]. Prediabetic hyperglycemia, diabetes, and hypertension are reported to contribute to CKD in about 75% of patients [7]. Patients with CKD are at increased risk of uncontrolled/resistant hypertension, which requires multiple medications to reach normal blood pressure ranges [8]. Uncontrolled/resistant hypertension is reported in approximately 23% of patients with CKD [9], and is particularly prevalent in patients with stage 4 CKD [10, 11].

Mineralocorticoid receptors (MR) and the role of their activation in inflammation, kidney damage, fibrosis, and CKD progression have received a great deal of recent research attention. MR are found in a variety of tissues, including the kidney, central nervous system, and heart [12–14]. Steroidal MR antagonists (MRAs) such as spironolactone and eplerenone are used to treat hypertension and heart failure [15]. In combination with angiotensin receptor blockers or angiotensin-converting enzyme inhibitors, these MRAs have been shown to decrease albuminuria in patients with CKD; however, adverse effects can include hyperkalemia, hypotension, and acute kidney injury, and these medications are therefore not typically used in patients with advanced CKD [16, 17].

Ocedurenone (KBP-5074) is a novel, oral, nonsteroidal, selective MRA under investigation for the treatment of uncontrolled/resistant hypertension in advanced CKD [18]. It selectively binds human MR with much less binding affinity to glucocorticoid, progesterone, or androgen receptors. A recent phase 2b clinical trial (BLOCK-CKD, ClinicalTrials.gov Identifier: NCT03574363) found that ocedurenone may be an effective and safe treatment for patients with stage 3b/4 CKD and uncontrolled/resistant hypertension due in part to its low risk of hyperkalemia [19]. However, for ocedurenone to be considered a viable treatment for patients with CKD and uncontrolled/resistant hypertension, it is important to determine its pharmacokinetics and any drug–drug interactions (DDIs) because it will be used in addition to other antihypertensive drugs and is primarily metabolized by CYP 3A enzymes.

The US Food and Drug Administration (FDA) provides clear guidance for the proper conduct of in vitro [20] and clinical [21] drug interaction studies for CYP enzyme- and transporter-mediated drug interactions. In vitro experiments are conducted to ascertain the role of CYP enzymes and transporters in the clearance of a drug, assess the influence of CYP enzymes and transporters on a drug's disposition, and determine the impact of the drug on CYP enzymes and transporters [20]. Specifically, the FDA recommends evaluation of induction and inhibition of the major CYP enzymes, including CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 [20]. It also recommends evaluation of the induction and inhibition of transporters that can significantly affect drug absorption, distribution, and elimination or have their expression or activity modified by a drug, including P-glycoprotein [P-gp or Multi-drug resistance 1 (MDR1) protein], breast cancer resistance protein (BCRP), organic anion transporting polypeptide 1B1 and 1B3 (OATP1B1/3), organic cation transporter 1 and 3 (OAT1/3), multidrug and toxin extrusion proteins 1 and 2-K (MATE1/2-K), and organic cation transporter 1 and 2 (OCT1/2) [20]. Developing an understanding of whether and how a drug interacts with these transporters can clarify the clinical consequences of altered drug tissue distribution. P-gp and BCRP are both expressed in tissues throughout the gastrointestinal tract, kidney, brain, and liver, and can therefore affect tissue distribution, oral bioavailability, and renal and hepatic elimination [20]. OATP1B1/3 act as uptake transporters in the sinusoidal membrane of hepatocytes, while renal transporters OAT1/3 and OCT1/2 (found in the basolateral membrane of the renal proximal tubule) and MATE1/2-K (expressed on the brush border membrane) play a role in active renal drug secretion [20].

In combination with clinical pharmacokinetic data, the results of these in vitro studies are then used to determine the utility and design of clinical DDI studies [20]. Clinical DDI studies with a sensitive index substrate are warranted if a drug is found to be an inhibitor or inducer of metabolizing enzymes or if it is found to act as a substrate, inducer, or inhibitor of drug transporters, with consideration given to the drug's safety margin and therapeutic index and other medications that the target patient population is likely to take [20].

In accordance with this guidance, the objectives of this study were as follows:

1. To identify which enzymes are involved in the phase I metabolism of ocedurenone;
2. To establish whether ocedurenone is an inhibitor or inducer of metabolizing enzymes CYP1A2, 2C8, 2C9, 2C19, 2D6, and/or 3A4;
3. To ascertain the substrate characteristics of ocedurenone for efflux transporters P-gp and BCRP and uptake transporters OATP1B1/3, OAT1/3, OCT1/2, and MATE1/K-2;

4. To determine the inhibitory potential of ocedurenone on major drug transporters P-gp, bile salt export pump (BSEP), BCRP, MDR1, OATP1B1/3, OAT1/3, OCT1/2, and MATE1/K-2; and finally, dependent on the results of these in vitro studies,

5. To investigate the effect of co-administration of a CYP3A4 inhibitor (itraconazole) and CYP3A4 inducer (rifampin) on the plasma pharmacokinetics of a single dose of ocedurenone.

2 Materials and Methods

2.1 In Vitro Studies

2.1.1 Test Compounds and Materials

Ocedurenone and KBP-5314 (internal standard) were prepared at KBP Biosciences,. Pooled human plasma was collected from healthy participants (Shanghai Center for Drug Metabolism and Pharmacokinetics Research, China). Human liver microsomes (HLM, Lot No.: 34689), human recombinant CYP1A2 (Lot No.: 30974), CYP1B1 (Lot No.: 68977), CYP2A6 (Lot No.: 33769), CYP2B6 (Lot No.: 49736), CYP2C8 (Lot No.: 03459), CYP2C9 (Lot No.: 00621), CYP2C19 (Lot No.: 69449), CYP2D6 (Lot No.: 75311), CYP2E1 (Lot No.: 73026), CYP3A4 (Lot No.: 16022), CYP3A5 (Lot No.: 44743), and CYP4A11 (Lot No.: 31489) were all purchased from BD Gentest™ (USA). Human primary hepatocytes were purchased from XenoTech (Lots H949 and HC2-8, Kansas, USA) and In Vitro Technologies (Lot OHO, MD, USA). Suppliers of materials used in the in vitro assays for relevant drug-metabolizing enzymes or transporters are listed in Table S1. Other reagents were commercially available and of special reagent grade, high-performance liquid chromatography grade, liquid chromatography–mass spectrometry grade, or equivalent. Further details of in vitro materials and methods can be requested from KBP Biosciences.

2.1.2 Characterization of Ocedurenone Metabolism

Ocedurenone was incubated in mixed HLM and recombinant CYP to identify the enzymes involved in phase I metabolism. For the recombinant enzyme test, the incubation system consisted of 100 mM of phosphate-buffered saline (PBS, pH 7.4), 3 μm of ocedurenone, and 2 mM of nicotinamide adenine dinucleotide phosphate (NADPH). Following a 3-minute pre-incubation period, reactions were initiated by adding the recombinant proteins (50 pmol/L). At 60 min, the reactions were terminated by adding 0.20 mL of ice-cold acetonitrile. Human liver microsomes inactivated with heat were used as a negative control. For the HLM test, experiments were performed in the presence or absence of chemical enzyme inhibitors (1-aminobenzotriazole or ketoconazole) in pooled HLM to determine the relevance of individual pathways. The incubation (200 μL) contained 100 mM PBS, 1.0 mg/mL microsomal proteins, 3 μm ocedurenone, and 2 mM NADPH. Reactions were initiated by the addition of ocedurenone. Metabolites in recombinant enzyme and HLM incubation systems were measured with ultra-high-performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF MS) methods.

2.1.3 In Vitro P450 Inhibition

The stock solutions of the probe substrates phenacetin (50.0 mM), paclitaxel (20.0 mM), tolbutamide (100 mM), S-mephenytoin (100 mM), dextromethorphan (10.0 mM), midazolam (5.00 mM), and testosterone (50.0 mM) were prepared using methanol and diluted with phosphate buffer (100 mM, pH 7.4) before being added to the incubation system. The organic solvents in the final incubation were no more than 0.20%. The incubation medium was 100 mM PBS, and the final incubation volume was 200 μL, containing 25 pmol/mL recombinant CYP enzyme, probe substrate, 1 mM NADPH, and ocedurenone at 0.5, 1, 5, 10, 25, 50, or 200 μM. For the CYP2C9 testing system, PBS was replaced with Tris buffer. The reactions were initiated after 5 min of pre-incubation at 37 °C and were terminated by the addition of 200 μL ice-cold acetonitrile. The enzyme concentration and incubation time were in the linear range. The reaction times used for CYP1A2, 2C8, 2C9, 2C19, 2D6, and 3A4 were 50 min, 20 min, 60 min, 35 min, 15 min, and 10 min or 35 min, respectively. All the incubations were conducted in duplicate, and the samples were stored at – 80 °C until use. The inhibitory effects of ocedurenone on CYP1A2, 2C8, 2C9, 2C19, 2D6, and 3A4 were investigated, and the corresponding IC_{50} values (the concentration of inhibitor required to achieve 50% inhibition) were measured. The enzyme activities were reflected by the number of metabolites formed. The percentage of remaining activity in the presence of ocedurenone was plotted against ocedurenone concentration using Presim. IC_{50} values were generated by a probit model with SPSS 17.0.

2.1.4 In Vitro P450 Induction

Human hepatocytes from three donors were seeded on 48-well collagenated plates for 24 h before treatment with the test article. The cells were co-incubated with ocedurenone at 0.1, 1, and 10 μM for 72 h, and rifampin (20 μM) and omeprazole (100 μM) were used as positive inducers for CYP3A4 and 1A2; the culture medium was changed daily during incubation. After incubation, the medium

was discarded and replaced with 200 μL phenol red-free culture medium containing 100 μM phenacetin (CYP1A2 probe substrate) or 200 μM testosterone (CYP3A4 probe substrate). After incubation in the presence of the probe substrates at 37 °C for 1 h, the supernatants were collected and stored at − 80 °C until analysis. The generated metabolites of the CYP1A2 and CYP3A4 probe substrates were analyzed with liquid chromatography with tandem mass spectrometry (LC-MS-MS). The induction effects of the positive control inducers and ocedurenone were assessed by the amount of metabolites formed in the induced and uninduced hepatocytes. Test compounds were considered inducers when the percent induction of positive control equaled or exceeded 40%. The percent induction of positive control was calculated using the following equation: percent induction of positive control (%) = (test sample activity – negative control activity) / (positive control activity – negative control activity) × 100. Calculation of the standard deviation and the percent induction of positive control was completed with Microsoft Excel 2003.

2.1.5 Substrate Characteristics of Ocedurenone for Drug Transporters

Substrate characteristics of ocedurenone for the efflux transporters P-gp and BCRP were investigated using parental and BCRP- or MDR1-transfected Madin-Darby Canine Kidney (MDCKII) cell monolayers. Four concentrations (0.06, 0.6, 6, and 60 μm) of ocedurenone were set for efflux transporter substrate experiments. Probe substrates and reference inhibitors were used for positive and negative controls, respectively, to determine efflux ratios (ER). The substrate potential of ocedurenone for the uptake transporters was determined using MDCKII or human embryonic kidney (HEK293) cells stably expressing the respective uptake transporters, including MATE1, MATE2-K, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, and OCT2. Standard inhibitors and probe substrates were used. Four concentrations (0.04, 0.4, 4.0, and 40 μm) of ocedurenone were set for uptake transporter substrate experiments.

2.1.6 Inhibitory Potential of Ocedurenone on Major Drug Transporters

Inhibition of the efflux transporters, P-gp, BSEP, and BCRP by ocedurenone was assessed at concentrations of 0.47–40 μM with inside-out membrane vesicles prepared from HEK293 cells overexpressing human ATP-binding cassette (ABC) transporters. Human solute carrier (uptake) transporters were evaluated in transfected MDCKII or HEK293 cells at concentrations of 0.63–40 μM using selective probe substrates and reference inhibitors. Probe

substrate transport was assessed in the absence and presence of ocedurenone, and the IC_{50} values were determined.

2.2 Phase 1 Clinical Drug–Drug Interaction Study

2.2.1 Study Design and Participants

This was a phase 1, open-label, parallel, 2-arm, fixed-sequence study, conducted from October 7, 2020 to December 3, 2020 at Covance (Madison, WI, USA). The clinical DDI was designed to investigate the effect of co-administration of a CYP3A4 inhibitor (Cohort 1, itraconazole multiple dose) and CYP3A4 inducer (Cohort 2, rifampin multiple dose) on the plasma pharmacokinetics of a single dose of ocedurenone in 24 healthy male and female participants aged 18–60 years with a body mass index of 18–32 kg/m². There were 12 subjects in each cohort.

2.2.2 Study with Strong CYP3A Inhibitor and Inducer

Following a 26-day screening period, participants (12 subjects) in Cohort 1 received a single oral dose of 0.5 mg ocedurenone after an overnight fast of at least 8 h on day 1. On day 15, an oral dose of 200 mg itraconazole solution was administered twice daily after an overnight fast of at least 8 h before the morning dose and a 2-h fast before the evening dose. From day 16 to 18, an oral dose of 200 mg itraconazole solution was administered once daily after an overnight fast of at least 8 h. On day 19, a single dose of 0.5 mg ocedurenone and a single dose of 200 mg itraconazole solution was given after an overnight fast of at least 8 h. From day 20 to 23, a single oral dose of 200 mg itraconazole solution was given after an overnight fast of at least 8 h. On days 1 and 19, serial blood samples (4 mL each) were collected pre-dose, and at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 168, and 240 h post-dose). Plasma was separated and plasma concentrations of ocedurenone were analyzed using a validated LC/MS/MS method (Fig. 1).

For participants (12 subjects) in Cohort 2, a single oral dose of 0.5 mg ocedurenone was administered after an overnight fast of at least 8 h on day 1. From day 15 to 20, a single oral dose of 600 mg rifampin was given after an overnight fast of at least 8 h. On day 21, a single dose of 0.5 mg ocedurenone and a single dose of 600 mg rifampin were co-administered after an overnight fast of at least 8 h. From day 22 to 25, an oral dose of 600 mg rifampin was given after an overnight fast of at least 8 h. On days 1 and 21, serial blood samples were collected pre-dose and at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 168, and 240 h post-dose) for analysis of plasma concentrations of ocedurenone (Fig. 2).

Concentrations of ocedurenone in human plasma (dipotassium ethylene diamine tetraacetic acid, K_2EDTA) were determined using a validated LC-MS-MS method,
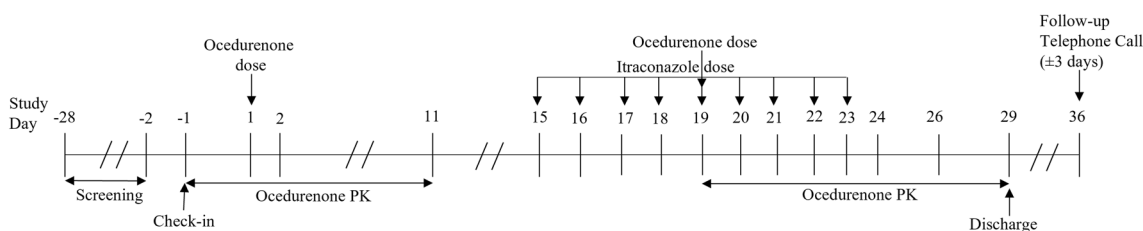
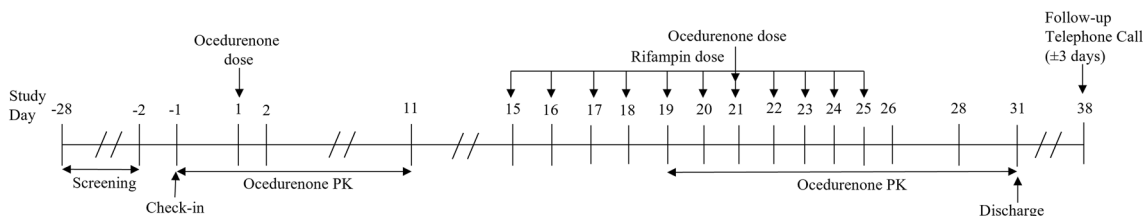**Fig. 1** Study schematic: Cohort 1; *PK* pharmacokinetics

Fig. 2 Study schematic: Cohort 2; *PK* pharmacokinetics

which utilized an API-5500 mass spectrometer (Applied Biosystems/MDS SCIEX) and Shimadzu Prominence 20 Series HPLC system. Ocedurenone was extracted from 50 µL human plasma (K_2EDTA) by protein precipitation using 250 µL acetonitrile (ACN) containing 0.1% formic acid (FA). After centrifuging, 200 µL of supernatant was evaporated to dryness under a stream of nitrogen at 40 °C. Samples were reconstituted by adding 150 µL of ACN:H_2O:FA (45:55:0.1). Extracts were kept refrigerated until analysis by LC-MS-MS. The analyte and internal standard were eluted using water: FA (100:0.1, V:V) and ACN:FA (100:0.1, V:V) with a 2.5-min gradient method on a C18 column (ACE 5, 50 × 2.1, 5 µm). Selective detection of ocedurenone was performed in multiple reaction monitoring and positive ionization modes by monitoring the transitions of m/z 504.2→306.1 and 490.3→306.1, respectively. The lower quantification limit for ocedurenone in this method was 0.1 ng/mL, and the linear range of determination was 0.1–100 ng/mL (correlation coefficient > 0.995). The intra-day precision of this method at lower limit of quantitation (LLOQ), and at low, medium, and high concentrations ranges from 1.1% to 5.8%, and intra-assay accuracy ranges from − 2.0% to 12.3%. The inter-assay precisions ranged from 2.5% to 6.6%, and overall accuracy ranged from 2.0% to 6.0%.

2.2.3 Ethics

The clinical DDI trials were conducted according to the protocol and according to consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, applicable International Conference on Harmonization Good Clinical Practice guidelines, and applicable laws and regulations. The protocol, informed consent, and other relevant study documentation were approved by the appropriate institutional review board at each participating site. All participants provided written informed consent in accordance with federal and institutional guidelines.

2.2.4 Safety Assessment

During this study, the safety-related outcomes of the subjects—clinical laboratory evaluations (clinical chemistry, hematology, and urinalysis), vital sign measurements, 12-lead electrocardiograms (ECGs), and physical examination. and adverse events (AEs)—were collected and evaluated. An AE was defined as any untoward medical occurrence. All AEs experienced by the subjects were graded according to the National Institutes of Health—Common Terminology Criteria for Adverse Events (NCI-CTCAE) v.4.03. The relationship of each AE to the study drug was graded as either not related, unlikely related, possibly related, or related. A serious adverse event (SAE) was defined as any untoward medical occurrence that at any dose resulted in death, was life-threatening, required inpatient hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions), resulted in a congenital anomaly/birth defect, or resulted in an important medical event.

Fig. 3 Possible metabolites of ocedurenone formed in human liver microsome incubation (**a**) in the presence of 1-aminobenzotriazole (**b**), and ketoconazole (**c**) detected by ultra-high performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF MS) methods
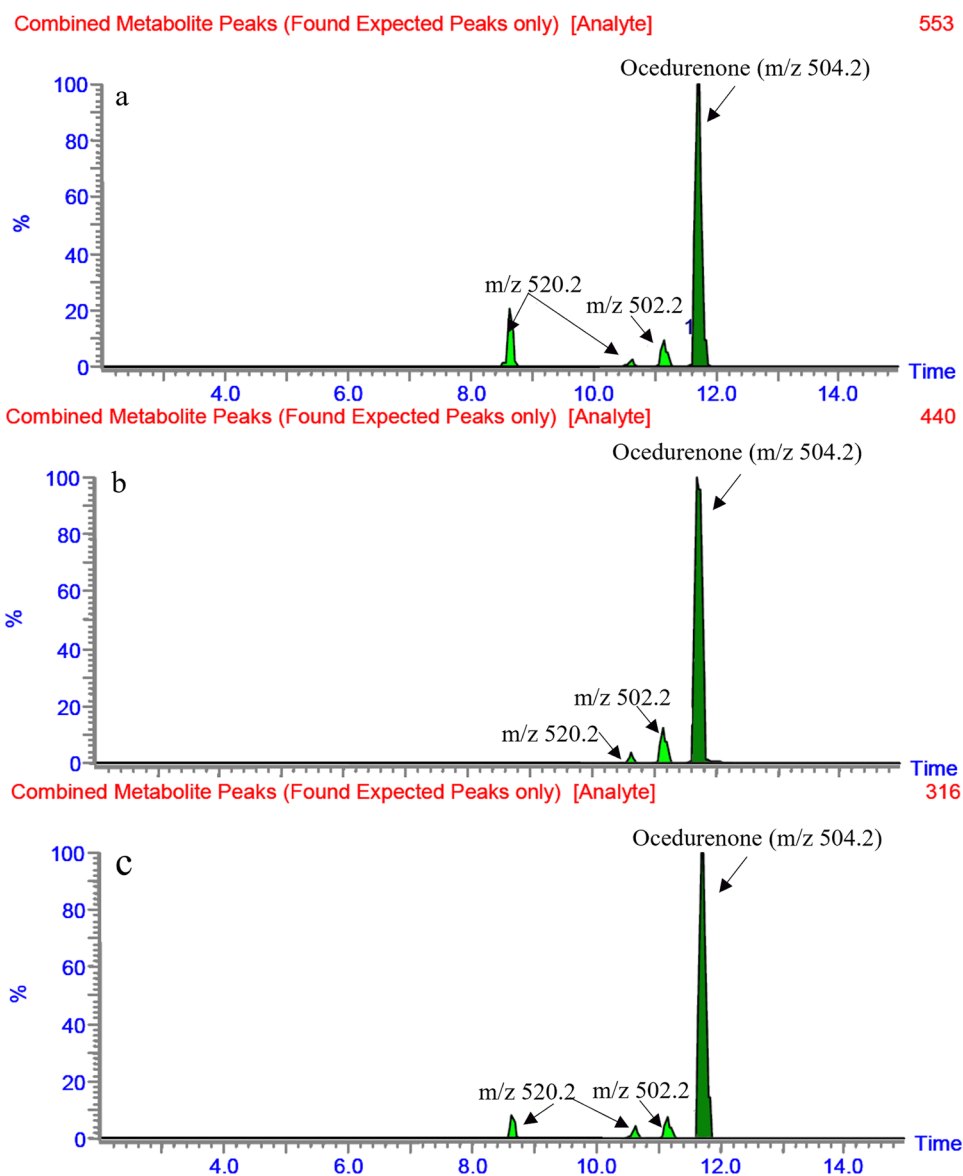
2.2.5 Statistical Analysis

Pharmacokinetics analyses were performed using noncompartmental methods using Phoenix WinNonlin (Certara v.8.1) to determine the ocedurenone pharmacokinetic parameters: area under the plasma concentration–time curve to the last measurable plasma concentration (AUC_{last}), area under the plasma concentration–time curve extrapolated to infinity (AUC_{0-inf}), peak plasma concentration (C_{max}), time to peak plasma concentration (T_{max}), half-life ($t_{1/2}$), oral clearance (CL/F), and apparent volume of distribution during terminal phase (Vz/F). Data statistical analysis was performed using the SAS® statistical software package v.9.4. Plasma concentrations and pharmacokinetics parameters for ocedurenone were summarized by cohort, treatment, and nominal time using descriptive statistics. For characterization of ocedurenone single-dose pharmacokinetics, parameters for ocedurenone 0.5-mg treatment in Cohorts 1 and 2 were pooled for summary statistics. For DDI assessments, the primary pharmacokinetics parameters were AUC_{0-inf}, AUC_{last}, and C_{max} for ocedurenone on each of the ocedurenone dosing days in both Cohorts 1 and 2. All other pharmacokinetics parameters were regarded as secondary and were not subject to inferential statistical analysis. A linear mixed-model analysis was applied to analyze the log-transformed primary pharmacokinetics parameters. The model assumed a fixed effect for treatment and a random effect for participant. Estimates of geometric mean ratios together with the corresponding 90% confidence intervals (CIs) were derived

for the comparisons of the pharmacokinetics parameters as follows: ocedurenone plus itraconazole multiple dose (test) versus ocedurenone alone (reference) for Cohort 1; ocedurenone plus rifampin multiple dose (test) versus ocedurenone alone (reference) for Cohort 2.

Based on the analysis of ln-transformed data, the point estimates and 90% CIs for the least-squares mean difference between treatments [(day 1) – (day 19 OR 21)] were exponentiated to obtain point estimates and 90% geometric CIs for the ratio [(day 1) / (day 19 OR 21)] on the original scale. No clinically significant change in ocedurenone exposure was concluded if the 90% geometric CI of the ratio [(day 1) / (day 19 OR 21)] of least-squares means from the analysis of variance of the ln-transformed AUC_{last}, AUC_{0-inf}, and C_{max} were entirely within the standard equivalence range of 80–125%. Interactions were described as strong, moderate, or weak if AUC alterations were > 5-fold, 2- to 5-fold, or 1.25- to 2-fold, respectively.

3 Results

3.1 In Vitro Evaluations

3.1.1 Characterization of Ocedurenone Metabolism

Ocedurenone was relatively stable in different recombinant CYP isoenzymes. Only minor metabolism was detected in incubations with CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP4A11. Most extensive metabolism was identified in incubations of CYP3A4.

In the HLM incubations, chemical inhibitors 1-aminobenzotriazole and ketoconazole were added separately into the HLM incubations. Ocedurenone metabolism was inhibited, suggesting that ocedurenone is likely metabolized by CYP3A4 (Fig. 3).

3.1.2 In Vitro Inhibition and Induction Potential of Ocedurenone on Human Cytochromes P450

According to the reported criteria (IC_{50} >10 μM categorized as weak inhibition, 1 μM < IC_{50} < 10 μM categorized as medium inhibition, IC_{50} <1 μM categorized as potent inhibition) [22], ocedurenone had little or no inhibition on CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 and weak inhibition on CYP2C8. Standard curves and regression equations were used for quantification of the metabolites. Fitted curves are shown in Fig. 4. The percent remaining activity of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 (midazolam-based), and CYP3A4 (testosterone-based) after co-incubation with ocedurenone at 200 μM were 92.9%, 70.2%, 78.5%, 87.8%, 70.2%, and 68.1%, respectively, and the IC_{50} was > 200 μM. The percentage remaining activity of CYP2C8 after co-incubation with ocedurenone at 200 μM was 26.4% and the IC_{50} was > 10 μM.

The activities of the three batches of human primary hepatocytes after treatment of the positive control (omeprazole or rifampin) were 36.0-, 13.5-, and 141-fold greater

Fig. 4 Inhibition by ocedurenone of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 (1'-OH midazolam), and CYP3A4 (6β-OH testosterone). *CYP* cytochrome P450, *M* midazolam-based, *T* testosterone-based
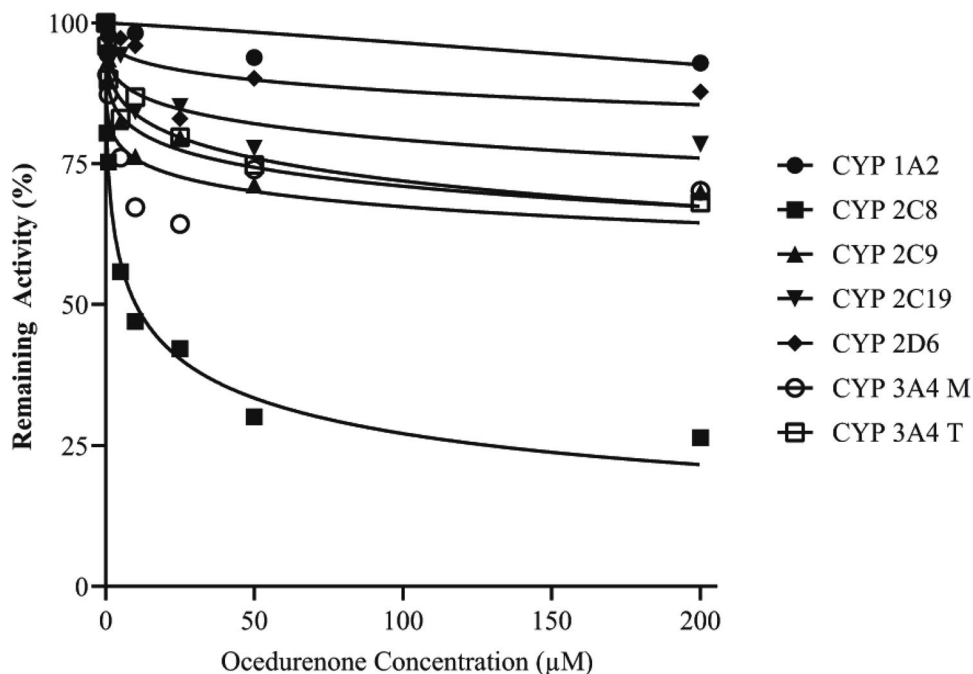
than the CYP1A2 activities of the negative control, and 12.8-, 3.36-, and 19.3-fold greater than the CYP3A4 activity of the negative control, suggesting that the test system was valid for evaluation of the induction effect of ocedurenone on CYP1A2 and CYP3A4. The percent CYP1A2 or CYP3A4 inductions of human primary hepatocytes after treatment with ocedurenone at 0.1, 1, or 10 μM were all lower than 40%, suggesting that ocedurenone had little or no induction on CYP1A2 or CYP3A4 in human primary hepatocytes [21].

3.1.3 Substrate Characteristics for Drug Transporters

In the bidirectional permeability measurement, ocedurenone showed higher permeability in the B–A direction than in the A–B direction, indicating that there was active transport of this compound in the MDCKII-BCRP and MDCKII-MDR1 cells. The highest observed efflux ratio in the case of the MDCKII-BCRP monolayer (net ER = ER_{BCRP}–ER_{MDCKII}) was 5.63 at 6 μM. The highest observed efflux ratio in the case of the MDCKII-MDR1 monolayer (net ER = ER_{MDR1}–ER_{MDCKII}) was 17.23 at 6 μM. The value of permeability and functional controls met the acceptance criteria, indicating that the MDCKII and MDCKII-BCRP/MDCKII-MDR1 cells were functioning properly. Ocedurenone is therefore likely a substrate of the human BCRP and P-gp transporters (Table 1).

In MDCKII or HEK293 cells stably expressing the respective uptake transporters (OATP1B1/3, OAT1/3, OCT1/2, and MATE1/K-2), accumulation of ocedurenone was similar in the transporter-expressing and the control cells (transporter-specific fold accumulations were < 2), indicating no active accumulation of the drug under all conditions tested. Ocedurenone is therefore not likely to be a substrate of any of the investigated uptake transporters (Table 1).

3.1.4 Inhibitory Potential of Ocedurenone on Major Drug Transporters

Ocedurenone inhibited all the investigated efflux transporters at 40 μM: BCRP with 98%, BSEP with 79%, and MDR1 with 99% (Table 1). The vesicular transport assays were repeated with 7 concentrations of ocedurenone to determine the IC_{50} values for the efflux transporters. Ocedurenone inhibited the BCRP-mediated transport in a dose-dependent manner with a maximum of 98% at 40 μm with an IC_{50} of 1.32 μM. Ocedurenone inhibited the BSEP-mediated transport with a maximum of 71% with an. estimated IC_{50} value of 16.38 μm with an applied bottom constraint to 0. Ocedurenone inhibited the MDR1-mediated transport with a maximum of 100% and a calculated IC_{50} value of 2.5 μm.

Ocedurenone inhibited the transporter-mediated probe substrate accumulation with more than 50% in the following transporters at 40 μM: MATE1, MATE2-K, OATP1B1,

Table 1 In vitro transport inhibition assays and substrate assays

Transporter and assay type	Inhibition results (IC_{50})	Substrate results		Probe substrate	Reference inhibitor
		Maximum net ER/fold accumulation	Substrate		
BCRP VT	1.3 μm	NA	NA	E3S (1 μm)	Ko143 (1 μm)
BSEP VT	16.4 μm[a]	NA	NA	TC (0.2 μm)	Cyclosporin A (10 μm)
MDR1 VT	2.5 μm	NA	NA	NMQ (1 μm)	PSC833 (1 μm)
BCRP ML	NA	5.63 at 6 μm	Likely	–	–
MDR1 ML	NA	17.23 at 6 μm	Likely	–	–
MATE1 UPT	6.7 μm	1.0	Not likely	Metformin (10 μm)	Pyrimethamine (1 μm)
MATE2-K UPT	7.9 μm[b]	1.3	Not likely	Metformin (10 μm)	Pyrimethamine (10 μm)
OAT1 UPT	NA	1.1	Not likely	$E_2$17βG (1 μm)	Rifampicin (50 μm)
OAT3 UPT	NA	1.2	Not likely	CCK-8 (0.1 μm)	Rifampicin (50 μm)
OATP1B1 UPT	21.9 μm	1.0	Not likely	PAH (5 μm)	Benzbromarone (300 μm)
OATP1B3 UPT	21.3 μm[a]	1.1	Not likely	E3S (1 μm)	Probenecid (500 μm)
OCT1 UPT	ND	1.1	Not likely	Metformin (10 μm)	Verapamil (100 μm)
OCT2 UPT	NA	1.0	Not likely	Metformin (10 μm)	Verapamil (300 μm)

[a]Constraint applied (bottom = 0). [b]Constraint applied (bottom = 0, top = 100).

BCRP breast cancer resistance protein, *BSEP* bile salt export pump, *CCK-8* cholecystokinin octapeptide, *$E_2$17βG* estradiol 17β-D-glucuronide, *E3S* estrone-3-sulfate, *MATE* multidrug and toxin extrusion transporter, *MDR1* multidrug resistance mutation 1, *ML* monolayer assay, *NA* not applied, *net ER* net efflux ratio, *NMQ* n-methyl quinidine, *OAT* organic anion transporter, *OATP* organic anion-transporting polypeptide, *OCT* organic cation transporter, *PAH* p-aminohippuric acid, *TC* taurocholate, *UPT* uptake assay, *VT* vesicular transport assay

OATP1B3, and OCT1 with maximum inhibition of 95%, 79%, 72%, 58%, and 49%, respectively. No interaction with the human OAT1, OAT3, or OCT2 transporters was observed. Where the maximum inhibition was higher than 50%, the inhibitory capacity of ocedurenone was assessed by determining IC_{50} values of the uptake transporters. Ocedurenone inhibited the investigated uptake transporters in the following order: MATE1 (IC_{50} = 6.7 μM), MATE2-K (IC_{50} = 7.9 μM), OATP1B3 (IC_{50} = 21.3 μm), and OATP1B1 (IC_{50} = 21.9 μm).

3.2 Phase 1 Clinical Drug Interaction Studies

Since in vitro studies identified ocedurenone as a substrate for CYP3A4 metabolism, the impact of co-administering inhibitors and inducers of these pathways was evaluated in a phase I clinical study. Co-administration of ocedurenone with a strong CYP3A inhibitor (itraconazole) and a CYP3A inducer (rifampicin) was investigated. The ocedurenone clinical DDI study was designed as a two-arm cross-over study. A total of 24 healthy volunteers were enrolled to participate with 12 volunteers in each cohort. In Cohort 1, ocedurenone (0.5-mg tablet) was dosed on study Days 1 and 19, and 200 mg itraconazole was given orally as 20 mL × 10-mg/mL solution twice daily on Day 15, followed by once daily dosing on Days 16–23 inclusive. In Cohort 2, 11 of 12 participants received ocedurenone (0.5-mg tablet) on study Days 1 and 21, and 600 mg rifampin was given orally as 2 × 300-mg capsules daily on Days 15–25 inclusive. One participant in Cohort 2 received 1 dose of ocedurenone 0.5 mg on Day 1 and 6

doses of rifampin 600 mg (total dose 3600 mg) on Days 15–20.

The arithmetic mean [+ standard deviation (SD)] plasma concentration profiles of ocedurenone following single oral dose administration of ocedurenone alone (Day 1, pooled from Cohorts 1 and 2), in combination with itraconazole (Day 19, Cohort 1), and in combination with rifampin (Day 21, Cohort 2) are presented in linear scale in Fig. 5. Summary pharmacokinetics parameters for ocedurenone are presented in Table 2.

3.2.1 Impact of Co-administered Itraconazole on the Pharmacokinetic Profile of Ocedurenone

The median T_{max} of 4.00 h (range: 2.00–6.00 h) following administration of ocedurenone alone was reduced to a median T_{max} of 2.00 h (range: 1.00–8.00 h) when ocedurenone was administered in combination with itraconazole. The geometric mean C_{max} of ocedurenone was approximately 1.1-fold higher when ocedurenone was administered in combination with itraconazole (C_{max} = 10.1 ng/mL) than when administered alone (C_{max} = 9.14 ng/mL). The plasma exposure of ocedurenone as measured by geometric mean AUC_{last} and AUC_{0-inf} was approximately 1.7-fold and 2.0-fold higher, respectively, when ocedurenone was administered in combination with itraconazole (AUC_{last} = 1090 h × ng/mL; AUC_{0-inf} = 1410 h × ng/mL) than when administered alone (AUC_{last} = 640 h × ng/mL; AUC_{0-inf} = 690 h × ng/mL). The observed higher plasma exposures of ocedurenone in the presence of itraconazole are consistent with the lower geometric mean CL/F and longer geometric

Fig. 5 Arithmetic mean (±SD) plasma concentrations of ocedurenone following single oral dose administration of ocedurenone 0.5 mg alone (Day 1, pooled), in combination with itraconazole (Day 19, Cohort 1), and in combination with rifampin (Day 21, Cohort 2) on a linear scale. *SD* standard deviation
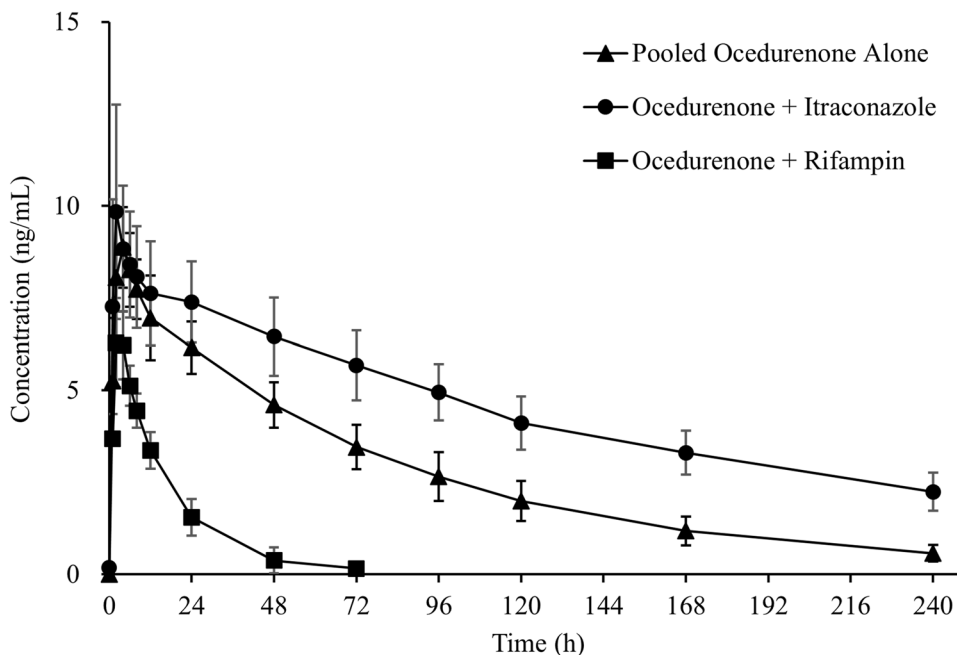
Table 2 Summary of the pharmacokinetic parameters of ocedurenone following single oral dose administration of ocedurenone 0.5 mg alone (Day 1, pooled), in combination with itraconazole (Day 19, Cohort 1), and in combination with rifampin (Day 21, Cohort 2)

Pharmacokinetic parameter	Ocedurenone ($n = 24$)	Ocedurenone + itraconazole ($n=12$)	Ocedurenone + rifampin ($n = 11$)
AUC_{last} (h × ng/mL)	640 (21.4)	1090 (16.7)	104 (16.8)
AUC_{0-inf} (h × ng/mL)	690 (23.8)	1410 (20.0)	109 (16.5)
C_{max} (ng/mL)	9.14 (18.2)	10.1 (23.2)	6.57 (11.3)
T_{max} (h)	4 (2.00–6.00)	2 (1.00–8.00)	4 (2.00–4.00)
$t_{1/2}$ (h)	60.3 (20.4)	125 (13.8)	10.9 (13.9)
CL/F (L/h)	0.725 (23.8)	0.355 (20.0)	4.61 (16.5)
Vz/F (L)	63.1 (15.1)	58.7 (17.8)	72.5 (9.70)

Geometric mean (CV) statistics presented; for T_{max} median (min–max) statistics presented

AUC_{0-inf} area under the plasma concentration–time curve from time zero extrapolated to infinity, AUC_{last} area under the plasma concentration–time curve from time zero to time of last quantifiable concentration (T_{last}), *CL/F* apparent total clearance, C_{max} maximum observed plasma concentration, *CV* coefficient of variation, *n* number of participants with valid observations, $t_{1/2}$ apparent terminal elimination half-life, T_{max} time of the maximum observed plasma concentration, *Vz/F* apparent volume of distribution

mean $t_{1/2}$ of ocedurenone in the presence of itraconazole (CL/F = 0.355 L/h; $t_{1/2}$ = 125 h) than of ocedurenone when administered alone (CL/F = 0.725 L/h; $t_{1/2}$ = 60.3 h). The geometric least squares mean ratios (test:reference) and 90% CIs for AUC_{last}, AUC_{0-inf}, and C_{max} for ocedurenone dosed in combination with itraconazole (test) and for ocedurenone dosed alone (reference) were 161.0 (152.3, 170.3), 199.5 (182.3, 218.3), and 107.4 (103.2, 111.8), respectively. None of the 90% CIs spanned unity, indicating that the effects of itraconazole on increasing ocedurenone exposure were statistically significant. The strong CYP3A inhibitor itraconazole had a weak effect (< 1.25-fold) on the exposure of ocedurenone.

3.2.2 Impact of Co-administered Rifampin on the Pharmacokinetic Profile of Ocedurenone

The median T_{max} of 4.00 h (range: 2.00–6.00 h) following administration of ocedurenone alone was similar to the median T_{max} of 4.00 h (range: 2.00–4.00 h) when ocedurenone was administered in combination with rifampin. The geometric mean C_{max} of ocedurenone was approximately 28% less when ocedurenone was administered in combination with rifampin (C_{max} = 6.57 ng/mL) than when administered alone (C_{max} = 9.14 ng/mL). The plasma exposure of ocedurenone as measured by geometric mean AUC_{last} and AUC_{0-inf} was approximately 84% less when ocedurenone was administered in combination with rifampin (AUC_{last} = 104 h × ng/mL; AUC_{0-inf} = 109 h × ng/mL) than when administered alone (AUC_{last} = 640 h × ng/mL; AUC_{0-inf} = 690 h × ng/mL). The observed lower plasma exposures of ocedurenone in the presence of rifampin are consistent with the higher geometric mean CL/F and shorter geometric mean $t_{1/2}$ of

ocedurenone in the presence of rifampin (CL/F = 4.61 L/h; $t_{1/2}$ = 10.9 h) than of ocedurenone when administered alone (CL/F = 0.725 L/h; $t_{1/2}$ = 60.3 h). The geometric least squares mean ratios (test:reference) and 90% CIs for AUC_{last}, AUC_{0-inf}, and C_{max} for ocedurenone dosed in combination with rifampin (test) and for ocedurenone dosed alone (reference) were 17.5 (16.6, 18.5), 17.2 (16.2, 18.2), and 73.4 (69.5, 77.5), respectively. None of the 90% CIs spanned unity, indicating that the effects of rifampin on decreasing ocedurenone exposure were statistically significant. The strong CYP3A4 inducer rifampin had a strong effect (> 5-fold) on the clinical pharmacokinetics of ocedurenone.

3.2.3 Safety Outcomes

Twenty-four subjects were enrolled in the study and were included in the safety analysis set; however, one subject in the Cohort 2 dose group was discontinued from the study early due to severe cholelithiasis and acute cholecystitis, which were not related to either ocedurenone or rifampin. There were no trends in the mean or individual subject clinical chemistry, hematology, coagulation, urinalysis, or lipid panel data during the study. No treatment-emergent AEs considered related to ocedurenone were reported by any subjects in Cohort 1. Two (16.7%) of 12 subjects in Cohort 1 experienced 5 treatment-emergent AEs considered related to itraconazole, which were mild in severity. Three of 12 (25.0%) subjects in Cohort 2 experienced 5 treatment-emergent AEs considered related to ocedurenone, which were mild in severity. All 12 (100%) subjects in Cohort 2 experienced 12 treatment-emergent AEs considered related to rifampin, which were mild in severity. Two SAEs reported

for 1 subject during the study in Cohort 2 were not related to either ocedurenone or rifampin.

4 Discussion

As a novel agent intended for use in patients with stage 3b/4 CKD and uncontrolled/resistant hypertension, a condition that necessitates polypharmacy, it is important to evaluate the risk of clinically relevant DDIs associated with the use of ocedurenone. In vitro testing indicated that ocedurenone was stable in human hepatic microsomes and that the metabolism of ocedurenone is predominantly catalyzed by CYP3A4. In vitro effects of ocedurenone on CYP enzyme activity further showed that ocedurenone had no inhibition on CYP1A2, 2C9, 2C19, 2D6, or 3A4 and weak inhibition on CYP2C8 with an IC_{50} value of 10 μm, and had little or no induction on CYP1A2 and 3A4 activity in human primary hepatocytes. Since the ocedurenone clinical dose was 0.25–0.5 mg daily, its plasma C_{max} was 32–40 ng/mL (0.06–0.08 μm) following 0.5-mg dose at steady state, it was reasonably anticipated that administration of ocedurenone would not result in pharmacokinetic DDI for drugs that the metabolism or clearance was CYPs-mediated. An in vitro substrate assay indicated that ocedurenone is likely to be a substrate for the efflux transporters P-gp and BCRP. Consequently, strong inhibitors of P-gp or BCRP may be expected to alter the pharmacokinetics of ocedurenone. As itraconazole inhibits P-gp, BCRP, and CYP3A4 while rifampin induces CYP3A4 and P-gp [22, 23], itraconazole and rifampicin were selected in the phase I clinical pharmacokinetic DDI study.

The doses of concomitant drugs used in this study (itraconazole 200 mg, rifampin 600 mg) are commonly prescribed and safely used for clinical DDI studies in healthy participants. A loading dose of 200 mg itraconazole twice daily or 400 mg once daily on Day 1 showed the highest inhibitory effects for itraconazole and is useful for compounds with long half-lives [23]. The Clinical Pharmacology Leadership Group recommends an itraconazole dosing regimen of 200 mg once daily with a 3-day run-in period prior to its coadministration with the substrate [24]. Therefore, administration in the fasted state of a loading dose of 200 mg twice daily followed by once daily for a total of 3 days prior to coadministration and 4 days following coadministration was planned for this study. A dose of 600 mg once-daily rifampin is recommended by the US FDA Guidance for Industry in evaluating the potential drug interactions that involve CYP3A4 induction [21]. Maximal simulated induction of intestinal CYP3A4 was achieved faster than that of hepatic CYP3A4, such that 90% maximal induction of intestinal CYP3A4 was achieved in 100% of the cohort within 5 days, whereas at least 9 days of rifampin dosing was required to achieve 90% maximal induction in the liver. Furthermore,

following cessation of rifampin, 90% of the cohort returned to within 25% of baseline intestinal CYP3A4 activity within 5 days. Therefore, administration of rifampin once daily for a total of 6 days prior to coadministration and 4 days following coadministration was planned for this study [25].

The fixed-sequence design used in this study is typical for interaction studies for which a relatively small number of participants is required because it allows for intraparticipant comparisons and reduced interparticipant variability. This study was open label because the study endpoints were not considered to be subjective. Ocedurenone was given as a single oral dose, which was expected to provide an appropriate assessment of the impact of the probe drugs on ocedurenone pharmacokinetics. Conducting the study in healthy participants mitigated the potential confounding effects of the disease state and concomitant medications.

In a Phase 1, first-in-human, single ascending dose study, ocedurenone doses from 0.5 to 30 mg administered orally were found to be safe and well-tolerated [22]. The intended marketed doses are 0.25 and 0.5 mg. The planned ocedurenone dose of 0.5 mg in this study was high enough to fully characterize the single oral dose pharmacokinetics of the parent compound. Ocedurenone displays low systemic clearance and a long half-life when administrated alone in healthy volunteers.

Administration of ocedurenone in combination with the CYP3A4 inhibitor itraconazole increased the plasma exposure of ocedurenone approximately 1.1- to 2.0-fold based on AUC_{last}, AUC_{0-inf}, and C_{max} compared to administration of ocedurenone alone. None of the 90% CIs spanned unity, indicating that the effects of itraconazole on increasing ocedurenone exposure were statistically significant.

Administration of ocedurenone in combination with rifampin decreased the plasma exposure of ocedurenone by approximately 84% based on AUC_{last} and AUC_{0-inf} and by approximately 28% based on C_{max} compared to administration of ocedurenone alone. None of the 90% CIs spanned unity, indicating that the effects of rifampin on decreasing ocedurenone exposure were statistically significant. A reduction in the level of ocedurenone exposure combined with a shorter $t_{1/2}$ is indicative of an increase in metabolism, systemic clearance, or both due to the induction of CYP3A4 by rifampicin.

The strong CYP3A4 inhibitor had a weak effect on ocedurenone exposure while the strong CYP3A4 inducer had a strong effect on ocedurenone exposure. In the itraconazole cohort, there were no treatment-emergent adverse events (TEAEs) considered to be related to ocedurenone. In the rifampin cohort, 3 of 12 participants (25.0%) experienced 5 TEAEs considered by the investigator to be related to ocedurenone. However, all TEAEs were mild in severity, including nervous system disorders [headache in 2 (16.7%) of 12 participants], gastrointestinal disorders [nausea in 1 (8.3%)

of 12 participants], psychiatric disorders [nervousness in 1 (8.3%) of 12 participants], and skin and subcutaneous tissue disorders [hyperhidrosis in 1 (8.3%) of 12 participants]. One adverse event unrelated to either ocedurenone or rifampin led to 2 serious adverse events reported for 1 participant during the study in Cohort 2. This participant was discontinued from the study early due to severe cholelithiasis and acute cholecystitis. Otherwise, there were no other clinically significant findings in the clinical laboratory evaluations, vital sign data, 12-lead ECG, or physical examination data during the study.

The results of a multiple ascending dose study in healthy participants and participants with mild to moderate renal impairment demonstrated that ocedurenone was safe and generally well-tolerated at doses of 2.5 mg and 5.0 mg once daily for 14 days in healthy participants, and at doses of 0.5 mg and 2.5 mg once daily for 56 days in participants with mild to moderate renal impairment [26]. Therefore, ocedurenone may allow greater opportunity for general use in combination with other medications with less cause for concern regarding DDI.

Ocedurenone had little inhibition or induction effect on CYP3A4 in human hepatic microsomes or primary hepatocytes. In addition, there were very low levels of oxidative metabolites detected in plasma and urine samples (unpublished data). Intestinal CYP3A4 could be induced faster than that of hepatic CYP3A4 when rifampicin is administrated. Fecal excretion is the predominant route of elimination for ocedurenone in humans following a single oral dose of 5 mg containing approximately 100 μCi of [14C]-ocedurenone (unpublished data). It seems that intestinal CYP3A4 might play an important role in the clearance of ocedurenone. Given that CYP3A4 induction may decrease ocedurenone plasma exposure, a more detailed understanding of the relationship between ocedurenone exposure and clinical effects is desirable, and would help determine the clinical impact of CYP3A4 induction on ocedurenone efficacy. Preclinical evaluation has also shown that ocedurenone may have the potential to inhibit intestinal efflux transporters such as P-gp and BCRP. However, ocedurenone has no potential to inhibit P-gp or BCRP in vivo, as $I_{gut}/IC_{50} < 10$ (I_{gut}/IC_{50} was 2.64 for BCRP and 1.58 for P-gp). The exposure changes observed during concomitant inhibitor or inductor administration seem to be mainly due to elimination rate changes of ocedurenone.

In the phase I clinical studies in healthy participants, ocedurenone was safe and well-tolerated when administered alone or in combination with multiple doses of itraconazole or rifampicin. Taken together, these in vitro and clinical studies demonstrate that ocedurenone has a low potential for drug–drug interactions.

5 Conclusions

In summary, the in vitro study results showed that ocedurenone had no inhibition on CYP1A2, 2C9, 2C19, 2D6, or 3A4, weak inhibition on CYP2C8 with an IC_{50} of 10 μm, and had little or no induction on CYP1A2 and 3A4 activity in human primary hepatocytes. Since the ocedurenone clinical efficacious dose was 0.25–0.5 mg daily, its plasma C_{max} was 32–40 ng/mL (0.06–0.08 μm) following a 0.5-mg dose at steady state, it was reasonably anticipated that administration of ocedurenone would not result in DDI that was CYP enzyme-mediated. However, since ocedurenone metabolism appeared to be CYP3A-mediated, we completed a clinical DDI study to evaluate the potential effects of a strong CYP3A inhibitor itraconazole and a CYP3A inducer rifampin on the pharmacokinetics of ocedurenone. Study results indicated that itraconazole had a weak effect on ocedurenone exposure, AUC_{0-inf} increased 104%, while rifampin co-administration resulted in very significant reduction in ocedurenone exposures. AUC_{0-inf} reduced 84% from 690 h×ng/mL to 109 h × ng/mL, and mean C_{max} reduced 28% from 9.54 ng/mL to 6.17 ng/mL, in the absence and presence of rifampin co-administration. Ocedurenone was well-tolerated when administered as a single 0.5-mg dose both alone and in combination with itraconazole or rifampin.

Declarations

applicable laws and regulations. The study was approved by WCG IRB (Study#: 8450126; Approval Date October 13, 2020).

Consent for Participate Informed consent was obtained from all individual participants included in the study.

Consent for Publication Not applicable.

Availability of Data and Materials The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code Availability Custom software or code used during the study are available from the corresponding author on reasonable request.

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