Metabolism and Disposition of Verinurad, a Uric Acid Reabsorption Inhibitor, in Humans[§]

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ABSTRACT

Verinurad (RDEA3170) is a second generation selective uric acid reabsorption inhibitor for the treatment of gout and asymptomatic hyperuricemia. Following a single oral solution of 10-mg dose of [¹⁴C]verinurad (500 μ Ci), verinurad was rapidly absorbed with a median time to occurrence of maximum observed concentration (T_{max}) of 0.5 hours and terminal half-life of 15 hours. In plasma, verinurad constituted 21% of total radioactivity. Recovery of radioactivity in urine and feces was 97.1%. Unchanged verinurad was the predominant component in the feces (29.9%), whereas levels were low in the urine (1.2% excreted). Acylglucuronide metabolites M1 (direct glucuronidation) and M8 (glucuronidation of N-oxide) were formed rapidly after absorption of verinurad with terminal half-life values of approximately 13 and 18 hours, respectively. M1 and M8

constituted 32% and 31% of total radioactivity in plasma and were equimolar to verinurad on the basis of AUC ratios. M1 and M8 formed in the liver were biliary cleared with complete hydrolysis in the GI tract, as metabolites were not detected in the feces and/or efflux across the sinusoidal membrane; M1 and M8 accounted for 29.2% and 32.5% of the radioactive dose in urine, respectively. In vitro studies demonstrated that CYP3A4 mediated the formation of the N-oxide metabolite (M4), which was further metabolized by glucuronyl transferases (UGTs) to form M8, as M4 was absent in plasma and only trace levels were present in the urine. Several UGTs mediated the formation of M1, which could also be further metabolized by CYP2C8. Overall, the major clearance route of verinurad is metabolism via UGTs and CYP3A4 and CYP2C8.

Introduction

Gout is a metabolic condition that, in most patients, is attributed to inadequate uric acid excretion leading to hyperuricemia and subsequent deposition of urate crystals in tissues of the body. These crystals can form in and around the joints (resulting in recurrent attacks of inflammatory arthritis) and kidney vasculature and tissues (resulting in decreased kidney function and the formation of kidney stones). In 2013, the estimated 12-month prevalence of chronic gout was approximately 6.3 million people in Europe (France, Germany, Italy, Spain, and the United Kingdom), 6.2 million people in the United States, and 2.8 million people in Japan (Heap and Sosa, 2012). Gout is approximately three times more common in men than in women (Zhu et al., 2011) with the greatest gender disparity in subjects under the age of 60. However, the disparity lessens as the prevalence in women increases with age (Mikuls et al., 2005; Bhole et al., 2010). The treatment of gout requires both acute and chronic management in which rapid pain relief is needed for acute gout attacks, and long-term treatment is achieved by keeping serum uric acid (sUA) levels below 6 mg/dl to dissolve the monosodium

urate crystal deposits (Bardin and Richette, 2014; Diaz-Torne et al., 2015).

First line treatment of gout utilizes urate-lowering therapy (ULT), allopurinol or febuxostat, which are inhibitors of xanthine oxidase (XOI). Clinical trials have shown that many patients fail to attain target sUA using a single ULT alone (Becker et al., 2005, 2015; Singh et al., 2015). In 2015, lesinurad (ZURAMPIC), an inhibitor of the uric acid reabsorption transporter 1 (URAT1), was approved in combination with an XOI to treat chronic gout and asymptomatic hyperuricemia for patients who fail to attain target sUA using a single ULT alone. URAT1 is responsible for the reabsorption of filtered uric acid from the renal tubular lumen. By inhibiting URAT1, lesinurad increases uric acid excretion and thereby lowers serum uric acid. The combination of lesinurad and a xanthine oxidase inhibitor provides gout patients with a new therapy that works to reduce the production of uric acid as well as to increase its renal excretion, thereby lowering sUA to a greater extent than xanthine oxidase inhibitor alone (Perez-Ruiz et al., 2011, 2016; Shen et al., 2011; Saag et al., 2014, 2015).

Verinurad (formerly RDEA3170), a second generation selective uric acid reabsorption inhibitor that inhibits URAT1, is being developed for the treatment of chronic gout and asymptomatic hyperuricemia. Verinurad is currently in phase 2 development. In comparison with

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ABBREVIATIONS: AME, absorption, metabolism and excretion; AUC, area under the curve; AUC_∞, area under the plasma concentration-time curve from zero to infinity; C_{max}, maximum observed concentration; CRU, clinical research unit; HLM, human liver microsomes; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LLOQ, lower limit of quantification; LSC, liquid scintillation counting; P450, cytochrome P450; SRM, selected reaction monitoring; sUA, serum uric acid; T_{max}, time to occurrence of maximum observed concentration; UDPGA, uridine diphosphate glucuronic acid; UGT, glucuronyltransferase; URAT1, uric acid reabsorption transporter 1.

lesinurad, verinurad shows improved potency toward URAT1 (25 nM vs. 7.3 μ M) in the kidney proximal tubules, thereby increasing the excretion of uric acid and lowering sUA (Tan et al., 2011, 2017; Girardet and Miner, 2014; Diaz-Torne et al., 2015), as well as improved half-life compared with lesinurad (13 hours vs. 6 hours respectively) (Shen et al., 2017). Like lesinurad, verinurad is highly protein bound with free fraction of 2%. Owing to the greater potency of verinurad, the proposed effective therapeutic dose of verinurad is significantly lower than that for lesinurad (10 mg compared with 200 mg, respectively) (Bardin et al., 2015; Shen et al., 2017).

To date, verinurad has been evaluated in single- and multiple-dose ascending clinical studies with favorable safety and tolerability, and with linear pharmacokinetics up to and including 40 mg following single-dose (Shen et al., 2017). Slight accumulation [$\sim$ 30% maximum observed concentration ($C_{\max}$) and area under the curve (AUC)] was observed when verinurad was dosed at 10 mg. Reported herein is the absorption, metabolism, and excretion of a single 10-mg oral dose of [14 C]verinurad in eight healthy male subjects. The aims of the study were to assess the disposition of verinurad, identify and quantify the exposure of circulating metabolites in human plasma, determine the metabolite profiles in excreta, and understand the enzymes involved in the metabolic disposition of verinurad in humans.

Materials and Methods

Radiolabeled Drug and Reagents

Unlabeled verinurad (2-(3-(4-cyanonaphthalen-1-yl)pyridin-4-ylthio)-2-methylpropanoic acid) for clinical studies was synthesized at Piramal Pharma Solutions (Torcan, Canada) following the synthetic route described in the patent (Ouk et al., 2013). [14 C]verinurad was synthesized by Moravex Biochemicals (Brea, CA), and method is provided in the Supplemental Material. The four 14 C atoms are present in the naphthalene ring (see Fig. 1). The radiochemical purity of [14 C]verinurad was 99.3% and the specific activity of the isolated compound was 57.1 mCi/mmol. Verinurad acyl glucuronide (M1), verinurad N-oxide (M4), verinurad N-oxide acyl glucuronide (M8), and [D_6]-stable labeled metabolites for M1, M4, and M8 for clinical analysis were synthesized by Syncom BV (Groningen, The Netherlands). Verinurad, M1, M4, and M8 for in vitro studies were synthesized by Ardea Biosciences (San Diego, CA). Synthesis of metabolites are described in the Supplemental Material.

Recombinant human P450 and glucuronyl transferases (UGTs) (Supersomes) and ultra-pooled human liver microsomes (HLM) for P450- and UGT-reaction phenotyping were purchased from Corning Life Sciences (Tewksbury, MA). Pooled HLM for determination of UGT and P450 contributions and Michaelis-Menten kinetics was purchased from Sekisui Xenotech (Lenexa, KS). All other materials were of high-performance liquid chromatography (HPLC) or analytical grade.

Clinical Study

The clinical study was conducted at Covance Clinical Research Unit (Madison, WI) and approved by institutional review board (Schulman associates IRB, Cincinnati, OH). This was a Phase 1, open-label, single-dose absorption, metabolism, and excretion (AME) study in healthy adult male subjects. Eight healthy adult male subjects received a single 10-mg dose of verinurad on day 1 in the fasted state. Verinurad was provided as an oral solution (0.34 mg/g verinurad prepared in trisodium citrate dehydrate and water) containing [14 C]verinurad (15.61 μ Ci/g), with each subject receiving approximately 500 μ Ci (on the basis of rat pharmacokinetic and dosimetry). Subjects were confined to the study site for a minimum of 4 days (96 hours postdose), and up to 13 days (312 hours postdose). Subjects were discharged on the basis of the following criteria: 1) a minimum of 90% of the administered radioactive dose was recovered in urine and feces, or 2) total recovery from urine and feces was less than 90%, and <1% of the administered radioactive dose was recovered in excreta on each of two consecutive days. If neither of the discharge criteria were met, subjects were discharged from the clinical research unit (CRU) on day 14 (312 hours post dose).

Fig. 1. Chemical structure of [14 C]verinurad.

Sample Collection

For pharmacokinetic analysis, blood samples were collected for determination of total radioactivity in whole blood and plasma, and plasma verinurad concentrations using two 10-ml tripotassium ethylenediaminetetraacetic acid (K_3 EDTA) Vacutainer-evacuated collection tubes. Samples were collected at the following time points in relation to dosing on day 1: predose (within 30 minutes prior to dosing); 15, 30, and 45 minutes, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 30, 36, 48, 54, 60, and 72 hours postdose, and subsequently at 24-hour intervals until the subject was discharged from the CRU. For metabolic profiling, an additional 20 ml of blood was collected on Day 1 predose, and at 1, 3, 6, 12, and 24 hours postdose. All blood samples were maintained on ice until centrifuged at 1500g for 10 minutes at 4°C within 45 minutes of collection. The separated plasma was divided equally, with half of the aliquots acidified by addition of one volume of 85% phosphoric acid to 100 volumes of plasma prior to storage. Samples were stored at approximately -70°C .

Urine samples were collected to determine urinary concentrations of verinurad and metabolites, total radioactivity, and metabolite profiling. Samples were collected at the following intervals in relation to dosing on day 1: -12 to 0, 0–6, 6–12, and 12–24 hours postdose, and subsequently pooled at 24-hour intervals until the subject was discharged from the CRU. The samples were refrigerated ($2-8^\circ\text{C}$) upon collection. Following completion of a collection interval, the samples were well mixed and aliquots (approximately 50 ml each) of urine were transferred into polypropylene storage tubes. For metabolite profiling, urine samples were acidified by addition of 0.5% of phosphoric acid (85%) (v/v) prior to storage. All urine samples were stored frozen at approximately -70°C until further analysis.

Fecal samples were collected to determine total radioactivity and metabolite profiling for the following intervals in relation to dosing on day 1: -12 to 0, 0–6, 6–12, and 12–24 hours postdose, and subsequently pooled at 24-hour intervals until the subject was discharged from the CRU.

For the determination of column recovery, extract of pooled plasma, pooled urine, and pooled feces homogenates were injected separately onto the HPLC-radioactive detection system with and without a column. HPLC column recovery was determined by comparing the total radioactivity of sample injected into the HPLC system with column to the total radioactivity of the same amount of sample without column analyzed by the online radiodetector, ν ARC Radio-LC system (AIM Research Company, Hockessin, DE).

Measurements of Total Radioactivity and Verinurad in Plasma, Urine and Feces

Total radioactivity was determined in blood, plasma, urine, and feces. Blood samples were mixed and combusted using a Sample Oxidizer Model 307 (Packard Instrument Company, Meriden, CT). The resulting $^{14}\text{CO}_2$ was trapped in Carbo-Sorb and mixed with PermaFluor (PerkinElmer) and then analyzed for radioactivity using a Model2900TR liquid scintillation counter (LSC; Packard Instrument Company) for at least 5 minutes or 100,000 counts. Plasma or urine samples were mixed with Ecolite (+) scintillation cocktail (MP Biomedicals, Santa Ana, CA) and analyzed directly by LSC. Fecal samples were combined at 24-hour intervals. A weighed amount (approximately three times the sample weight) of 1% phosphoric acid (85%) in 1:1 ethanol/water (v/v) was added to the fecal samples and then homogenized using a probe-type homogenizer. The sample was then combusted (as described for blood) and analyzed by LSC. For all

matrices, analysis was performed in duplicate weighed aliquots (approximately 0.2 g).

For quantitative determination of verinurad in plasma and in urine, 40- μ l aliquots of unacidified plasma samples or 25 μ l of unacidified urine were precipitated with 200 μ l acetonitrile containing [D₆]verinurad (2 ng/ml) as internal standard (IS). The samples were vortexed for 5 minutes at maximum speed. Samples were then centrifuged at 3300 rpm for 10 minutes at 4°C. The supernatant (150 μ l) was transferred to a 1-ml Nunc 96-well plate containing 150 μ l water and mixed. Sample volume of 20 μ l was injected for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Chromatographic separation was performed on an Agilent 1100 liquid chromatographic system (Agilent Technologies, Santa Clara, CA) running a mobile phase gradient consisting of A) water with 0.1% formic acid (v/v, aqueous phase) and B) acetonitrile with 0.1% formic acid (v/v, organic phase), at a flow rate of 0.9 ml/min through a Zorbax SB-C18, 4.6 $\times$ 50 mm, 3.5 μ m HPLC column (Agilent Technologies) for plasma or Synergi Polar-RP, 4.6 $\times$ 50 mm, 4 μ m HPLC column (Phenomenex, Torrance, CA) for urine samples. The gradient from 0 to 2 minutes was 50% A and 50% B, 2 to 3 minutes was 5% A and 95% B, 3–3.01 minutes was 50% A and 50% B, and maintained at 50% A and 50% B to 5 minutes. The column effluent was delivered to an API 5000 mass spectrometer (AB Sciex, Framingham, MA) operated in positive TurboIonSpray mode. Mass spectrometric analysis of verinurad and IS was done in selected reaction monitoring (SRM) mode with the precursor to product ion transitions of m/z 349 $\rightarrow$ 263 and 355 $\rightarrow$ 264, respectively, dwell times of 150 milliseconds for each transition, and at unit mass resolution for the first quadrupole and low mass resolution for the third quadrupole. A dummy ion transition was included with a 30 milliseconds dwell time to reduce potential for crosstalk. Methodology was validated according to the US FDA Bioanalytical Method Validation (BMV) Guidance (2001). The lower limit of quantification (LLOQ) for verinurad in plasma and urine was 0.100 and 2 ng/ml, respectively.

Plasma, Urine, and Feces Sample Preparation for Metabolite Profiling

Acidified plasma samples from 1, 3, 6, 12, and 24 hours postdose were thawed on ice. Per time point, aliquots of 0.5 ml from each subject were pooled together for a final volume of 4 ml. The pooled plasma was extracted with 8 ml of chilled acetonitrile. The precipitated proteins were removed by centrifugation at 3660 rpm (Allegra 6R Centrifuge; Beckman Coulter, Jersey City, NJ) and 5°C for 15 minutes. The supernatant fractions were transferred to glass tubes, dried down under N₂ stream at room temperature, and reconstituted with acetonitrile/water mixture (1:2) for HPLC injection.

Acidified urine samples were thawed on ice and urine samples collected from 0–6, 6–12, and 12–24 hours were pooled together as a single 0–24 hour sample from all eight subjects according to equal percentage (by weight) of each sample. Similarly, urine samples collected from 24 to 48 hours were thawed on ice and pooled together from all eight subjects to form a single 24- to 48-hour sample. Other urine samples were not processed for metabolite profiling owing to sample radioactivity less than 1% of dose. The pooled 0- to 24-hour urine sample was analyzed by HPLC with radioactive detection and mass spectrometric detection without further sample preparation. The pooled 24- to 48-hour sample was concentrated down to approximately 50% under N₂ stream at room temperature before HPLC injection.

The feces homogenates from each subject collected from 0 to 120 hours postdose were thawed on ice and pooled by 24-hour interval according to approximately equal percentage (by weight) of each fecal sample. Samples with radioactivity less than 1% of dose were excluded from pooling. The pooled feces homogenates were centrifuged at $\sim$ 3000g for 15 minutes at 4°C. The supernatant fraction was transferred to a separate container. The precipitates were extracted with approximately two sample volumes of ethanol/water (2:1) containing 1% of phosphoric acid (v/v). After vortexing for 30 seconds, the mixture was centrifuged again. The extraction step was repeated once more. The supernatant fractions were combined, concentrated down under N₂ stream at room temperature, and then filtered through 0.22- μ m cellulose acetate membrane prior to HPLC injection.

HPLC radioanalyses of urine, plasma, and feces extracts was carried out using the Q-TRAP API4000 LC-MS/MS system coupled to ν ARC Radio-LC system. Separation of verinurad and its metabolites following a 100- μ l injection was achieved by using a reverse phase C8 analytical column (Agilent Eclipse XDB-C8 5 μ m, 4.60 $\times$ 250 mm) running a mobile phase gradient consisting of water with 0.1% formic acid (v/v, aqueous phase) and acetonitrile with 0.1% formic acid (v/v,

organic phase), at a flow rate of 1.0 ml/min. Run time was 45 minutes. The gradient from 0 to 3 minutes was 90% A and 10% B, 3–30 minutes was 90% A and 10% B, 30–35 minutes was 5% A and 95% B, 35–39 minutes was 5% A and 95% B, 39–39.5 minutes 90% A and 10% B, and maintained at 90% A and 10% B to 45 minutes.

Quantitation of Metabolites (M1 and M8) in Plasma and Urine for Pharmacokinetic Analysis

Prior to the ¹⁴C-AME metabolite processing, M1 was found to be stable in plasma and urine without acidification. For quantification of M1 in plasma, 100- μ l aliquots of plasma were precipitated with 0.500 ml of acetonitrile with 0.2% formic acid, v/v, containing 0.600 ng/ml internal standard ([D₆]verinurad-M1, M1-IS). The supernatant was evaporated and the remaining residue reconstituted with 0.150 ml of 0.2% formic acid in 1:9 acetonitrile/water (v/v). For urine samples, 50 μ l was precipitated with 0.150 ml of acetonitrile with 0.2% formic acid, v/v, containing 200 ng/ml M1-IS. A 25- μ l aliquot of the supernatant was then diluted with 0.2 ml of 0.2% formic acid in water, v/v. Sample volume of 20 μ l (plasma extract) or 10 μ l (urine extract) was injected for LC-MS/MS analysis. Chromatographic separation was performed on an Agilent 1100 liquid chromatographic system (Agilent Technologies) running a mobile phase gradient consisting of water with 0.1% formic acid (v/v, aqueous phase) and acetonitrile with 0.1% formic acid (v/v, organic phase), at a flow rate of 0.9 ml/min through a Kinetex C18, 4.6 $\times$ 50 mm, 2.6 μ m HPLC column (Phenomenex). The gradient from 0 to 5 minutes was 80% to 70% A, then from 5 to 6 minutes was 70% to 5% A, then maintained at 5% A for 1 minute, and finally back to 80% A at 7.01 minutes. The column effluent was delivered to an API 5000 mass spectrometer (AB Sciex) for plasma or API 4000 mass spectrometer (AB Sciex) for urine operated in positive TurboIonSpray mode. Mass spectrometric analysis of M1 and M1-IS was done in SRM mode with the precursor-to-product ion transitions of m/z 525 $\rightarrow$ 263 and 531 $\rightarrow$ 263, respectively, dwell times of 200 milliseconds for M1 and 70 milliseconds for M1-IS, and at unit mass resolution for the first quadrupole and low mass resolution for the third quadrupole. A dummy ion transition was included with a 30-millisecond dwell time. Under reverse-phase chromatographic conditions, both compounds separate into two diastereomeric peaks, which were both used for quantitation (i.e., both chromatographic peaks were integrated and then summed). Methodology was validated according to the US FDA BMV Guidance (2001). The LLOQ for M1 in plasma and urine was 0.05 and 10.0 ng/ml, respectively.

For quantification of M8, human plasma and urine samples were acidified with phosphoric acid to limit acyl-migration and back conversion of M8 to its aglycone M4, an *N*-oxide metabolite of verinurad. Because M4 levels were not present in plasma and negligible amounts present in urine, an indirect assay was developed to quantify M8 through total M4. Fifty microliters of acidified plasma samples was alkalinized with 20 μ l of 2 M potassium hydroxide solution and incubated at room temperature to completely convert M8 to its aglycone counterpart (M4). The samples were then precipitated with 0.200 ml of 2% formic acid in acetonitrile, v/v, containing 4.00 ng/ml [D₆]verinurad-M4 as internal standard (M4-IS) and 80 μ l of supernatant was then diluted with 0.200 ml water. Sample volume of 20 μ l was injected for LC-MS/MS analysis. Likewise, 25 μ l of acidified urine samples were alkalinized with 10 μ l of 2 M potassium hydroxide solution and incubated at room temperature to completely convert M8 to M4. The samples were then precipitated with 0.200 ml of 2% formic acid in acetonitrile, v/v, containing 250 ng/ml M4-IS and 20 μ l of the supernatants were then diluted with 0.280 ml of 1:2 acetonitrile/water. Sample volume of 10 μ l was injected for LC-MS/MS analysis. Chromatographic separation was performed on an Agilent 1100 liquid chromatographic system (Agilent Technologies) running a mobile phase gradient consisting of water with 0.1% formic acid (v/v, aqueous phase) and acetonitrile with 0.1% formic acid (v/v, organic phase), at a flow rate of 0.9 ml/min through a Kinetex C18, 4.6 $\times$ 50 mm, 2.6- μ m HPLC column (Phenomenex). The gradient from 0 to 2 minutes was 65%–5% A, then maintained at 5% A for 1 minute, and back to 65% A at 3.01 minutes. The column effluent was delivered to an API 5000 mass spectrometer (plasma) or API 4000 mass spectrometer (urine) (AB Sciex) operated in positive TurboIonSpray mode. Mass spectrometric analyses of M4 and M4-IS were done in SRM mode with the precursor to product ion transitions of m/z 365 $\rightarrow$ 279 and 371 $\rightarrow$ 280, respectively, dwell times of 150 milliseconds for M4 and 30 milliseconds for M4-IS, and at unit mass resolution for the first quadrupole and low mass resolution for the third quadrupole. A dummy ion transition was included with a 20-millisecond

dwell time. Methodology was validated according to the US FDA BMV Guidance (2001). The LLOQ for M4 in plasma and urine was 0.100 and 10.0 ng/ml, respectively. The concentration of M8 in the clinical plasma or urine sample (C_{G-M4}) was then obtained from the M4-total concentrations ($C_{M4-Total}$) according eq. 1.

The concentration of M8 in the clinical sample (C_{G-M4}) was then obtained from the M4-Total concentrations ($C_{M4-Total}$) according to the following equation:

$$C_{G-M4} \left(\frac{ng}{mL} \right) = C_{M4-Total} \left(\frac{ng}{mL} \right) \frac{540.54 \left(\frac{g}{mol} \right)}{364.42 \left(\frac{g}{mol} \right)} \quad (1)$$

Pharmacokinetic Calculation

Pharmacokinetic parameters were derived using Phoenix WinNonlin software, Version 6.3 (Pharsight Corporation, Mountain View, CA). The pharmacokinetic parameters [maximum observed concentration (C_{max}), time to occurrence of maximum observed concentration (T_{max}), area under the plasma concentration-time curve from zero to last quantifiable timepoint (AUC_{last}), area under the plasma concentration-time curve from zero to infinity (AUC_{∞}), oral clearance corrected by bioavailability F (CL/F), volume of distribution at equilibrium corrected by bioavailability F (V_{ss}/F), renal clearance (CL_R), and half-life ($t_{1/2}$)] for verinurad and metabolites were calculated from individual concentration-time profiles from radioactivity (whole blood and plasma) and LC-MS/MS (plasma or urine) using noncompartmental methods.

P450 Phenotyping with Recombinant P450s and Human Liver Microsomes and Chemical Inhibitors

P450 Isoform Incubations. Verinurad (10 μ M) was incubated with individual P450 enzymes (100 pmol/ml), FMO3 (0.5 mg/ml), or HLM (0.5 mg/ml) in a final volume of 200 μ l. The experiment was performed in triplicate and zero-time incubations served as negative controls. The mixture contained enzymes, potassium phosphate buffer (100 mM, pH 7.4), and magnesium chloride (3 mM). The reaction was initiated by the addition of NADPH (final concentration of 1 mM). The incubation at 37°C was stopped by the addition of 300 μ l of acetonitrile containing internal standard ([D₆]verinurad at 40 ng/ml) at 60 minutes.

Incubation with Human Liver Microsomes and Chemical Inhibitors. P450 confirmation studies were conducted in HLM and selective chemical inhibitor. Verinurad (1 μ M) was incubated with HLM (0.5 mg protein/ml) in a final volume of 200 μ l. The mixture contained HLM, potassium phosphate buffer (100 mM, pH 7.4), magnesium chloride (3 mM), and the CYP2C9 inhibitor sulfaphenazole (10 μ M) or CYP3A4 inhibitor ketoconazole (1 μ M). The experiment was performed in duplicate and zero-time incubations served as negative controls. The reaction was initiated by the addition of NADPH (final concentration of 1 mM) and incubated for 15, 30, 45, 60, and 90 minutes at 37°C. The reaction was terminated by the addition of 300 μ l of acetonitrile containing internal standard ([D₆]verinurad at 40 ng/ml).

UGT Isoform Profiling

UGT Supersome Incubation. To identify the UGT isoforms involved in the formation of M1, the following procedure was used. A 0.2-ml reaction mixture containing alamethicin (25 μ g/mg protein), UGT (0.5 mg/ml), uridine diphosphate glucuronic acid (UDPGA; 5 mM), magnesium chloride (10 mM), and [¹⁴H]verinurad (10 μ M, 2.5 μ Ci/ml) in 50 mM Tris (pH 7.4) was incubated at 37°C for 60 minutes. The experiment was performed in triplicate. Zero-time incubations served as negative controls. The reaction was initiated by the addition of UDPGA solution and terminated by the addition of 0.2 ml of chilled acetonitrile containing 100 ng/ml [D₆]verinurad M1 as internal standard. The precipitated proteins were removed by centrifugation at 3300g for 10 minutes, and the supernatant was analyzed by LC with radio- and mass-spectrometric detection for the formation of M1 metabolite.

To identify the UGT isoforms involved in the formation of M8 from M4, the following procedure was followed. A 0.5-ml reaction mixture with M4 (2 μ M), alamethicin (50 μ g/ml), D-saccharic acid-1,4-lactone (3 mM), UGT (0.5 mg/ml), UDPGA (2 mM), and magnesium chloride (10 mM) in 50 mM phosphate buffer (pH 7.4) was incubated at 37°C for 60 minutes. The experiment was performed in triplicate and incubations with UGT control Supersomes served as negative

controls. The reaction was initiated by the addition of UDPGA solution and terminated by the addition of 0.5 ml of ice-cold acetonitrile containing 100 ng/ml [D₆]verinurad M4 (internal standard) and 0.1% of formic acid.

Incubation with HLM and Chemical Inhibitor. To confirm the UGT isoforms involved in the formation of M1, an UGT chemical inhibition study was conducted. Currently, the availability of selective UGT inhibitor is limited and only UGT2B7 was confirmed using a chemical inhibitor. Fluconazole, a moderately selective competitive inhibitor of UGT2B7, was used to confirm the role of this isoform. Pooled HLM (2 mg/ml) was incubated with alamethicin (25 μ g/mg protein), UDPGA (5 mM), [¹⁴H]verinurad (10 μ M, 2.5 μ Ci/ml), MgCl₂ (10 mM), D-saccharic acid-1,4-lactone (5 mM), and fluconazole (5 mM) at 37°C for 60 minutes. The experiment was performed in triplicate. Zero-time incubations served as negative controls. The reaction was initiated by the addition of UDPGA (5 mM final concentration) solution and terminated by the addition of 200 μ l of ice-cold acetonitrile-containing [D₆]verinurad M1 (100 ng/ml) as an internal standard.

Chemical Stability of M1 and M8

The chemical stability of M1 and M8 in potassium phosphate buffer, pH 7.4 (KPB) was assessed at 37°C. KPB was prewarmed at 37°C for 5 minutes. The incubation was initiated with the addition of M1 or M8 at a final concentration of 10 μ M, final volume of 1 ml. An aliquot of 50 μ l was removed at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 hours and transferred to a 96-well HPLC plate well containing 50 μ l ice-cold acetonitrile containing internal standard M1-[D₆] and 2% formic acid. Samples were further diluted with the addition of 300 μ l of water and vortexed before LC-MS/MS analysis to determine concentration of M1 or M8 at each time point. The slope of the linear regression for the log-transformed concentration-versus-incubation time of M1 or M8 was used for the determination of the first-order degradation rate constant and half-life.

URAT1 Activity

HEK293 human embryonic kidney cells stably overexpressing human URAT1 were seeded into 96-well poly-D-lysine-coated tissue culture plates at a density of 1.5×10^5 cells per well and grown at 37°C and 5% CO₂ overnight. The next day the cell culture was washed once with wash buffer (125 mM sodium gluconate, 25 mM MES, pH 5.5). Compounds diluted in assay buffer (125 mM sodium gluconate, 4.8 mM potassium gluconate, 1.2 mM potassium phosphate monobasic, 1.2 mM magnesium sulfate, 5.6 mM glucose, and 25 mM MES, pH 5.5) with 1% dimethyl sulfoxide were preincubated with the cells (triplicate wells per condition) for 5 minutes at room temperature in a volume of 20 μ l, then 20 μ l of 200 μ M ¹⁴C-uric acid (American Radiolabeled Chemicals, St. Louis, MO) diluted in assay buffer was added to the plate and incubated for 10 minutes at room temperature. Free ¹⁴C-uric acid was removed by washing cells three times with Wash Buffer. Cells were lysed by adding 100 μ l of Ultima Gold scintillation fluid (PerkinElmer) to each well and radioactivity was counted using a MicroBeta2 plate reader (PerkinElmer).

For calculating percent inhibition, each plate contained triplicate wells of both the empty vector control and human URAT1 stable cells treated with vehicle (dimethyl sulfoxide) only. Using the cpm values obtained from the plate reader, percent inhibitions were calculated as follows:

$$\text{percent Inhibition} = 100 - ((100 * (\text{Test} - \text{Basal}) / (\text{Control} - \text{Basal}))) \quad (2)$$

where Basal and Control are the median cpm values of the vehicle treated control and URAT1 wells, respectively. IC₅₀ values were calculated using GraphPad Prism and the sigmoidal dose response (variable slope) equation.

LC-MS/MS Analysis to Support In Vitro Assays

For all in vitro experiments, following termination of incubation, the precipitated proteins were removed by centrifugation at 3300g for 10 minutes and the supernatant was collected for LCMS/MS analysis of analytes. An API 4000 triple quadrupole mass spectrometer, operated in positive TurbolonSpray mode, was used to monitor the precursor $\rightarrow$ product ion transitions shown below. Separation of analytes conducted by reverse-phase HPLC using an Agilent 1100 system connected to a Synergi Polar-RP 4- μ m column (Phenomenex) with mobile phase consisting of water with 0.1% formic acid (v/v, aqueous phase) and 0.1% formic acid in acetonitrile (v/v, organic phase); Table 1.

TABLE 1
Transition-phase parameters for HPLC to support in vitro assays

Analytes	MS/MS Transition (<i>m/z</i> , amu)
Verinurad	349 → 263
[D ₆]Verinurad	355 → 263
M4	365 → 279
[D ₆]Verinurad M4	371 → 279
M1	525 → 263
[D ₆]Verinurad glucuronide M1	531 → 263
M8	541 → 279
[D ₆]Verinurad glucuronide M8	547 → 279

amu, Atomic mass unit.

Results

HPLC Column Recovery and Sample Extraction Efficiency

Extraction efficiency of pooled plasma and feces samples was greater than 90%. The pooled urine samples were analyzed directly without sample extraction. HPLC column recovery was greater than 92% for plasma, urine, and feces samples, an indication of adequate recovery of radioactivity in the HPLC sample analysis.

Urinary and Fecal Recovery

Urine and fecal samples were collected up to 144 hours from eight subjects who met the discharge criteria. The cumulative excretion of radioactivity in urine and feces from all subjects was measured and is shown graphically in Fig. 2. An average ($\pm$ S.D.) $97.1\% \pm 1.58\%$ of the administered 10-mg dose of [¹⁴C]verinurad was recovered in total over the 144-hour collection period, with recovery in individual subjects ranging from 93.8% to 99.0%. Extraction efficiency of feces samples was greater than 90%.

Pharmacokinetics of Verinurad and Total Radioactivity

Verinurad was rapidly absorbed with median $T_{\max}$ of 0.5 hours postdose (Fig. 3) following oral administration. Verinurad exhibited a biphasic concentration-time profile. Plasma terminal half-life on the basis of total radioactivity was 35 hours (Table 2), whereas the plasma terminal half-life of verinurad was 15 hours (Table 2). Geometric mean AUC_{∞} values on the basis of total radioactivity in blood and plasma were

Fig. 2. Cumulative percent of radioactive dose recovered in urine and feces at specified intervals after administration of a single 10-mg (500 μ Ci) oral dose of [¹⁴C]verinurad.

556 and 906 nanogram equivalents $\cdot$ hours/ml, respectively. Blood-to-plasma ratios on the basis of total radioactivity for $C_{\max}$ and AUC_{∞} were 0.551 and 0.614, respectively (Table 2), indicating no preferential distribution of the drug into the red blood cells; the majority of circulating radioactivity was associated with plasma. The plasma verinurad-to-total radioactivity ratios for $C_{\max}$ and AUC were 0.464 and 0.213, respectively, indicating that metabolites were the predominant component in the circulation.

After absorption, verinurad plasma $C_{\max}$ and AUC_{∞} were 160 ng/ml and 193 ng $\cdot$ hour/ml, respectively (Table 2) on the basis of quantification via LC-MS/MS. Verinurad showed a moderate clearance of 51.8 l/h, $CL_{R0-96 \text{ hour}}$ of 11.5 ml/min, and a high volume of distribution (V_{ss}/F) of 504 l, suggesting extensive distribution of the drug to peripheral tissues.

In urine, 63.6% of the radioactive dose was recovered during the first 48 hours postdose (Table 3) with 64.5% of the radioactive dose recovered from 0 to 120 hour postdose, suggesting at least 64.5% of the dose was absorbed following oral drug administration (Fig. 2). The majority (>85%) of radioactivity recovered in the urine occurred in the first 24 hours postdose, and the fraction excreted at 24 hours postdose was 56% (Fig. 2). Only 1.3% of the dose was attributed to unchanged verinurad excreted in the urine up to 96 hours postdose. The renal clearance of verinurad was determined to be 11.5 ml/min based on the amount recovered in urine and plasma AUC .

In feces, unchanged verinurad was the predominant component (over 97% in sample radioactivity analysis) accounting for 29.9% of the dose by 120 hour post-dose (Fig. 2; Table 3), representing either unabsorbed drug and/or excreted via biliary elimination.

Metabolite Identification

Metabolite profiles of [¹⁴C]verinurad were determined in human plasma, urine, and feces (Fig. 4). Prior to the human [¹⁴C]-AME study reported herein, metabolite profile of [¹⁴C]-verinurad was conducted in both rat and dog. Six metabolites, oxidative and glucuronide conjugates, were identified in both species, which set the metabolite naming convention (data not shown). A total of three metabolites were identified in humans, including an acyl glucuronide (M1), an N-oxide (M4), and an acyl glucuronide of M4 (M8). The proposed metabolic pathway of verinurad and metabolites M1, M4, and M8 in healthy adult male subjects is depicted in Fig. 5 (see Supplemental Table 1 for chemical name).

Fig. 3. Mean (S.E.) concentration-time profiles of verinurad in plasma and total radioactivity in blood and plasma following a single oral dose of 10 mg oral solution with 500 μ Ci of [¹⁴C]verinurad (0–144 hours postdose). Only $n = 2$ and $n = 1$ samples were available, respectively, at 96 and 144 hours for total radioactivity in the plasma and blood.

TABLE 2

Summary of pharmacokinetic parameters (geometric mean, confidence interval 95%) of verinurad in blood and plasma following a single oral dose of [14 C]verinurad (10 mg, 500 μ Ci) given to healthy male subjects

Analyte	Matrix	T _{max} ^a	C _{max}	AUC _∞	CL/F	V _{ss} /F	t _{1/2}	CL _{r0-96 h}
		<i>h</i>	<i>ng/ml</i>	<i>ng·h/ml</i>	<i>l/h</i>	<i>l</i>	<i>h</i>	<i>ml/min</i>
Verinurad (N = 8)	Plasma	0.50 (0.50–0.75)	160 (117–219)	193 (152–246)	51.8 (40.7–65.9)	504 (368–690)	14.8 (11.9–18.4)	—
	Urine	—	—	—	—	—	—	11.5 (7.64–17.3)
Total radioactivity ^b (N = 8)	Plasma	0.50 (0.50–0.75)	344 (255–465)	906 (742–1110)	—	—	34.7 (26.9–44.7)	—
	Blood	0.50 (0.25–0.75)	190 (144–250)	556 (428–723)	—	—	37.5 (19.9–70.6)	—
	Blood-to-plasma ratio	—	0.551 (0.527–0.576)	0.614 (0.535–0.703)	—	—	—	—
	Plasma verinurad-to-total radioactivity	—	0.464 (0.433–0.496)	0.213 (0.194–0.234)	—	—	—	—

CL/F, oral clearance corrected by bioavailability F; t_{1/2}, half-life; V_{ss}/F, volume of distribution at equilibrium corrected by bioavailability F.

^aT_{max} is expressed as median (range).

^bC_{max} and AUC values expressed as nanogram equivalents/ml and nanogram equivalents · h/ml, respectively.

Plasma. Radioactivity was measurable in only the 1-, 3-, and 6-hour postdose pooled samples, as later time samples were below detection limits. Major metabolites M1 and M8 were observed and accounted for most of the circulating radioactivity representing 22%–31% and 35%–51% of plasma radioactivity, respectively, with no detection of M4 in the eight subjects.

Metabolite Profiling in Urine and Feces. The total radioactivity at 48 hours postdose excreted in urine and feces accounted for 63.6% and 30.6% of the dose, respectively (Table 3). M1 and M8 were exclusively eliminated in the urine, with recovered amounts of 29.2% and 32.5%, respectively, of the radioactive dose and M4 was present at trace levels (Table 3). In feces, unchanged verinurad was the predominant component. M1 and M8 were not found in the feces but trace levels of unidentified metabolites were observed (1.0% of the dose).

Pharmacokinetics of M1 and M8

Following verinurad administration, M1 and M8 were formed quickly with median T_{max} of 0.5–0.75 hours (Table 4). The majority (approximately 90%) of M1 and M8 exposure was cleared within 24 hours postdose.

The geometric mean plasma M1-to-verinurad ratio for C_{max} and AUC_∞ were 0.786 and 0.997, respectively. The geometric mean plasma M8-to-verinurad ratios for C_{max} and AUC_∞ were 0.485 and 0.943, respectively (Table 4). The AUC molar ratios of M1 and M8 to verinurad were both approximately 1:1. The plasma M1-to-total radioactivity ratios for C_{max} and AUC_∞ were 0.550 and 0.321, respectively. Similarly, plasma M8-to-total radioactivity ratios for C_{max} and AUC_∞ were 0.350 and 0.313, respectively. The elimination of M1 and M8 were only observed in the urine with high CL_{r0-96 hour} of 224 and 338 ml/min, respectively.

In Vitro Metabolism

Formation of M4 Metabolite. Incubation studies with recombinant P450s and FMO demonstrated that oxidative metabolism of verinurad to M4 was primarily mediated by CYP3A4 with a minor contribution by CYP2C9 and CYP3A5 (Fig. 6A). Studies conducted using HLM and selective chemical inhibitors ketoconazole and sulfaphenazole confirmed that CYP3A4/5 is the predominant oxidative enzyme with minor contribution by CYP2C9 (Fig. 7).

Formation of M1 and M8 Metabolites. Incubation studies with recombinant uridine 5'-diphospho-glucuronyl transferase (UGT) demonstrated that the formation of M1 was mediated by several UGTs, namely

UGT1A3, UGT2B4, UGT2B7, and UGT2B17 (Fig. 8A). As shown in Fig. 5, the formation of M8 can occur via two pathways. The formation of M8 via glucuronidation of M4 was mediated by several UGTs namely UGT1A1, UGT1A3, UGT2B7, UGT1A8, and UGT2B17 (Fig. 8B), although levels for UGT1A8 were low. Alternatively, the formation M8 can also occur via P450-mediated oxidation of M1. Recombinant P450 incubation studies showed that CYP2C8 was the major isoform involved in the oxidation of M1 via N-oxidation to form M8 (Fig. 6B). Confirmation studies were performed with HLM and quercetin, a selective CYP2C8 inhibitor (data not shown).

Buffer Stability of Acyl-Glucuronides

To assess the chemical reactivity of M1 and M8, in vitro studies were performed in 100 mM potassium phosphate buffer (KPB) at 37°C up to and including 24 hours. The half-life values of M1 and M8 in KPB were determined to be 7.65 and 3.51 hours, respectively.

Assessment of In Vitro URAT1 Inhibition by M1 and M8

M1 inhibition of URAT1 was evaluated in vitro and the average IC₅₀ was 2.9 ± 0.36 μ M (mean ± S.E., n = 10). Minimal inhibition of 14 C-uric acid uptake by URAT1 was observed with M8 and did not allow for IC₅₀ determination. Because both M1 and M8 were tested at concentrations well above the human C_{max} of 189 and 120 ng/ml (~350 and 220 nM, respectively), contribution to efficacy is low.

Discussion

The aim of this study was to investigate the disposition of verinurad in humans following an oral dose of [14 C]verinurad to eight healthy male

TABLE 3

Recovery of verinurad and metabolites in excreta as a percentage of verinurad dose

Pathway	Time Period (h)	% of Dose					
		M1	M4	M8	Others ^a	Verinurad	Total
Urine ^b	0–48	29.2	0.33	32.5	0.36	1.17	63.6 ^c
Feces ^b	0–120	ND	ND	ND	0.67	29.9	30.6
Total excreta ^d	0–120	29.2	0.33	32.5	1.03	31.1	94.2

ND, Not detectable.

^aOthers: including unidentified minor metabolites.

^bUrine collection up to 48 hours; and feces collection up to 120 hours.

^cUrine collection from 0 to 120 hours recovered 64.5% of dose.

^dThe recovery of total radioactivity from 0 to 144 hours interval was 97.1%.

Fig. 4. Verinurad and metabolites in pooled plasma, pooled urine, and pooled feces.

volunteers. Specifically, the study determined the mass balance of verinurad and its metabolites in circulation and excreta, and found that verinurad was well tolerated in all subjects with no significant adverse events reported. Verinurad was well absorbed with the extent of absorption estimated to be at least ~64% on the basis of urinary recovery of parent and metabolites.

The mean oral clearance (CL/F) was approximately 51.8 l/h, indicating moderate clearance. The radioactive dose was largely recovered in excreta (97.1% by 144 hours). Unchanged verinurad accounted for 29.9% of the dose excreted in the feces, with 1.3% of dose excreted unchanged in the urine, indicating that metabolism was the major route of elimination. The higher radioactivity in plasma relative to whole blood indicated minimal distribution of radioactivity to red blood cells. Furthermore, the large oral volume of distribution of 504 liters suggested that verinurad distributed extensively to peripheral tissues.

The verinurad-to-total radioactivity profiles indicated that metabolites were the predominant component in circulation, with AUC of M1 and M8 similar to that of verinurad. The formation of M1 occurred via direct glucuronidation by several UGT (UGT1A3, 2B4, 2B7, and 2B17) isoforms, whereas the formation of M8 occurred via a two-step process involving two distinct pathways. From in vitro studies, one pathway to M8 formation involved the oxidation of verinurad to M4 by CYP3A4 and to a lesser extent by CYP2C9 and CYP3A5, followed by efficient glucuronidation involving several UGT isoforms (UGT1A1, 1A3, 2B7, and 2B17) as M4 was not detected in circulation. The second pathway to M8 formation is the sequential oxidation of M1 by CYP2C8. Data generated herein suggest both pathways were plausible for formation of M8.

Interestingly, among all the CYP isoforms evaluated, only CYP2C8 was able to oxidize M1 to M8. Several glucuronide substrates have been identified as CYP2C8 ligands (Ma et al., 2017). Homology modeling

Fig. 5. The proposed metabolic pathway of verinurad in humans.

Fig. 6. Formation of M4 (A) and M8 (B) by recombinant P450s, FMO, and HLM.

has been used to examine substrate specificity of the CYP2C family (namely 2C8, 2C9, 2C18, and 2C19). Several key amino acids have been identified in the CYP2C8 active site that afford substrate selectivity, namely Ser114, Phe205, and Ile476, which enable binding of hydrophilic substrates like glucuronides to the active site (Ridderstrom et al., 2001; Schoch et al., 2004; Johnson and Stout, 2005). Several drugs, including gemfibrozil and clopidogrel, form acylglucuronides that are strong CYP2C8 time-dependent inhibitors (Ma et al., 2017). The CYP2C8 time-dependent inhibition of M1 and M8 was not investigated but will be in future studies.

Following oral administration of verinurad, the formation of acyl glucuronide metabolites occurred via first pass metabolism in the gastrointestinal (GI) tract and liver due to the presence of UGTs (Nakamura et al., 2008). Once verinurad is in the systemic circulation, formation of acylglucuronide metabolites may also occur in the kidney in addition to the liver. Interestingly, the pharmacokinetic data indicated that formation of M1 and M8 was rapid as $T_{\max}$ was reached within an hour.

The disposition of verinurad involved drug transporters (unpublished data), namely, active uptake transporters in the liver (OATP1B3) and

TABLE 4
Summary of pharmacokinetic parameters (geometric mean, confidence interval 95%) for M1 and M8

Analyte	$T_{\max}^a$	$C_{\max}$	AUC_{last}	AUC_{∞}	$t_{1/2}$	$CL_{0-96 \text{ h}}$
	<i>h</i>	<i>ng/ml</i>	<i>ng·h/ml</i>	<i>ng·h/ml</i>	<i>h</i>	<i>ml/min</i>
M1 (<i>N</i> = 8)	0.5	189	287	290	12.9	224
	(0.50–0.75)	(112–320)	(182–452)	(185–455)	(10.2–16.4)	(182–276)
M1-to-verinurad ratio	—	0.786	1.01	0.997	—	—
	—	(0.606–1.02)	(0.769–1.34)	(0.761–1.31)	—	—
M1-to-total radioactivity ratio	—	0.550	0.340	0.321	—	—
	—	(0.428–0.705)	(0.252–0.459)	(0.238–0.432)	—	—
M8 (<i>N</i> = 8)	0.75	120	273	283	18.1	338
	(0.50–0.75)	(102–142)	(227–329)	(235–342)	(12.4–26.5)	(286–399)
M8-to-verinurad ratio	—	0.485	0.936	0.943	—	—
	—	(0.381–0.617)	(0.769–1.14)	(0.777–1.14)	—	—
M8-to-total radioactivity ratio	—	0.350	0.324	0.313	—	—
	—	(0.282–0.433)	(0.290–0.361)	(0.279–0.351)	—	—

AUC_{∞} , area under the plasma concentration-time curve from zero to infinity; ($t_{1/2}$, half-life;

$^aT_{\max}$ is expressed as median (range).

Fig. 7. Inhibition of M4 formation in HLM by specific chemical inhibitors of CYP3A4 (ketoconazole) and CYP2C9 (sulfaphenazole).

kidney (OAT1/3), however, the data was inconclusive for OATP1A2 (GI tract). Verinurad has high permeability, so uptake across various organs involves both passive and active mechanisms. The disposition of verinurad and its acylglucuronide metabolites in the liver appeared to be dependent on both transport and metabolism involving enterohepatic recycling. This was evident by the lack of M1 and M8 in the feces, which suggested efficient hydrolysis by β -glucuronidase in the intestine releasing the aglycone for reabsorption to the systemic circulation. Enterohepatic recirculation was also evident by the secondary verinurad peak at 6 hours postdose (Fig. 3), which was consistent with the ex vivo findings that verinurad exhibited colonic absorption (unpublished data). Furthermore, studies conducted in bile duct-cannulated (BDC) rats also indicated enterohepatic recycling. In male BDC rats dosed with [14 C]-verinurad (10 mg/kg, oral), ~79% of the radiolabeled material, predominately M1, was eliminated in the bile (within 24 hours) and ~4%–11% was recovered in urine and feces. The bile collected from these animals (consisting mainly of M1) was then dosed via intra-duodenal infusion to a different group of rats; verinurad was the only component in the feces, indicating that M1 was converted efficiently to the aglycone in the GI tract (unpublished data).

The efflux of hepatic acylglucuronides across the sinusoidal membrane to the systemic circulation may be another mechanism followed by renal elimination, as the acyl glucuronides were detected in the urine but not the feces. In addition, the low fraction of verinurad excreted unchanged in the urine may be the result of kidney metabolism by UGTs, CYP3A5 and CYP2C8 though the expression level of CYP2C8 is not clear (Bieche et al., 2007). The excretion of M1 and M8 in the urine may be attributed in part (minor) to kidney metabolism in addition to hepatic metabolism (Nakamura et al., 2008; Knights et al., 2013). The renal clearance ($CL_{R0-96 \text{ hour}}$) of M1 and M8 of 224 and 338 ml/min, respectively, were both greater than the glomerulus filtration rate, indicating active efflux. Both M1 and M8 are substrates for MRP2 and MRP4 (data not shown) consistent with the active renal clearance.

Although M1 and M8 circulate in plasma at approximately 1:1 with verinurad, they are not pharmacologically active toward URAT1. Consequently, no further absorption, distribution, metabolism, and excretion characterization was performed from a substrate perspective but additional P450 and transporter inhibition studies will be conducted in the future. Although rat and dog do not make appreciable levels of M8 (unpublished data), this metabolite was present in rabbits and monkeys, providing the preclinical toxicology coverage required to support clinical studies.

Fig. 8. Formation of M1 (A) and M8 (B) metabolites by recombinant glucuronyl transferases. Data are mean ($\pm$ S.D.) of triplicate samples.

Acylglucuronides have been implicated in idiosyncratic drug toxicity due to their reactivity toward macromolecules (Smith et al., 1990). The buffer stability studies conducted with M1 and M8 indicated that they were stable, with half-life values of 7.65 and 3.51 hours, respectively. As per Sawamura et al. (2010), acylglucuronides exhibiting or exceeding a half-life of 3.6 hours in potassium phosphate buffer have a low risk of chemical instability. The half-life of M1 was well above this safe-drug criterion, but the half-life of M8 was similar to the criterion. The absence of an alpha hydrogen next to the carboxyl group in M1 or M8 can further reduce the reactivity of M1 or M8, thereby reducing the risk of idiosyncratic toxicity (Wang et al., 2004). Thus, the chemical stability data and the lack of an alpha hydrogen suggest a relatively low risk that M1 or M8 may cause idiosyncratic drug toxicity. This result was consistent with covalent binding studies performed in human hepatocytes, as the 10-mg clinical dose of verinurad fell within the safe zone (Nakayama et al., 2009) (data not shown). To date, hepatotoxicity observations related to verinurad have not been detected in clinical studies.

The potential for co-medications to clinically affect the pharmacokinetics of verinurad is low due to the involvement of several enzymes, CYP3A and UGTs. From the in vitro and in vivo investigations, glucuronidation may be the predominant pathway, however, a clinical study is needed to confirm this hypothesis. The clinical approach to deduce the dominant pathway maybe to conduct a CYP3A inhibition study as it may be difficult to inhibit all the UGT enzymes. To date, a medication known to broadly inhibit UGTs is not available. In general, UGTs are known to be high capacity enzymes with high K_m values ($> 10 \mu\text{M}$) (Kaivosaaari et al., 2011) which further suggest that it may be difficult to fully inhibit UGT mediated pathways. Thus, it is unlikely that

a co-medication can alter the metabolic disposition of verinurad due to the multiple pathways of elimination.

In conclusion, a single 10-mg dose of verinurad was safe and well tolerated in healthy male subjects. The human disposition of verinurad is predominantly via metabolism, although transporters also contribute. The metabolism of verinurad was primarily via UGTs and CYP3A4. Two acyl glucuronide metabolites (M1 and M8) circulate in concentrations equimolar to verinurad but lack efficacy toward URAT1. Furthermore, these acylglucuronides were characterized as being stable with low reactivity and low potential for idiosyncratic toxicity.

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Authorship Contributions

Participated in research design: Yang, Shen, Hall, Gillen.
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Contributed new reagents or analytic tools: Girardet.
Performed data analysis: Lee, Yang, Shah, Shen, Wilson, Ostertag, Hall, Gillen.

Wrote or contributed to the writing of the manuscript: Lee, Yang, Shen, Shah, Wilson, Ostertag, Gillen, Girardet.

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