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 \(^{14}C\)verinurad (500 µCi), 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 glucuronol 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 (Mikuls et al., 2005; Bhole et al., 2010). The treatment of gout requires 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; 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 (Mikuls et al., 2005; Bhole et al., 2010). 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lesinurad, verinurad shows improved potency toward URAT1 (25 nM vs. 7.3 μM) 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 [−30% maximum observed concentration (Cmax) 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 [14C]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

Radionabeled 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 (Thorcan, Canada) following the synthetic route described in the patent (Ouk et al., 2013). [14C]Verinurad was synthesized by Moravek Biochemicals (Brea, CA), and method is provided in the Supplemental Material. The four 14C atoms are present in the naphthalene ring (see Fig. 1). The radiochemical purity of [14C]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 D2546-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 glucuron 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 (Lexena, 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 [14C]verinurad (15.61 μCi/g), with each subject receiving approximately 500 μCi (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 (512 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).

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 (K3EDTA) 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°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 column to the total radioactivity of the same amount of sample without column analyzed by the online radiodetector, vARC 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 14CO2 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
Acidified plasma samples from 1, 3, 6, 12, and 24 hours postdose were thawed on ice. Per time point, an aliquot 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 3600 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 hour 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 radioisotope 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 ~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-µm cellulose acetate membrane prior to HPLC injection. HPLC radioanalyses of urine, plasma, and feces extracts were carried out using the Q-TRAP API4000 LC-MS/MS system coupled to a µARC Radio-LC system. Separation of verinurad and its metabolites following a 100-mg oral dose was validated according to the US FDA Biosanalytical 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. Acidified plasma samples from 1, 3, 6, 12, and 24 hours postdose were thawed on ice. Per time point, an aliquot 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. 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HPLC radioanalyses of urine, plasma, and feces extracts were carried out using the Q-TRAP API4000 LC-MS/MS system coupled to a µARC Radio-LC system. Separation of verinurad and its metabolites following a 100-µg injection was achieved by using a reverse phase C8 analytical column (Agilent Eclipse XDB-C8 5 µm, 4.60 × 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.

Quantitative 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-µl 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 (Da)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 µl was precipitated with 0.150 ml of acetonitrile with 0.2% formic acid, v/v, containing 200 ng/ml M1-IS. A 25-µl aliquot of the supernatant was then diluted with 0.2 ml of 0.2% formic acid in water, v/v. Sample volume of 20 µl (plasma extract) or 10 µl (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 × 50 mm, 5 µm HPLC column (Phenomenex). The gradient from 0 to 5 minutes was 80% A 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 → 263 and 531 → 263, respectively, dwell times of 200 milliseconds for M1 and 70 milliseconds for M1-IS, and at unit mass resolution for the third 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 0.055 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 alkalized with 20 µl 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 4.000 ng/ml Da verinurad-M4 as internal standard (M4-IS) and 80 µl of supernatant was then diluted with 0.200 ml water. Sample volume of 20 µl was injected for LC-MS/MS analysis. Likewise, 25 µl of acidified urine samples were alkalinized with 10 µl 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 µl of the supernatants were then diluted with 0.280 ml of 1:2 acetonitrile/water. Sample volume of 10 µl 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 × 50 mm, 2.6-µm HPLC column (Phenomenex). The gradient from 0 to 2 minutes was 90% A and 10% B, 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 TurbolonSpray 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 → 279 and 371 → 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, M8}) was then obtained from the M4-total concentrations (C_{M4 Total}) according to the equation:

\[
C_{G, M8} = \left( \frac{C_{M4, Total} \cdot 540.54}{364.42} \right)
\]

**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 (AUCC), area under the plasma concentration-time curve from zero to infinity (AUC∞), oral clearance by bioavailability F (CL/F), volume of distribution at equilibrium corrected by bioavailability F (V_{d}/F), renal clearance (CLR) CLR, 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 μM) 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 μl. 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 was stopped at 37°C with the addition of 300 μl of acetonitrile containing internal standard [D6]verinurad (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 μM) was incubated with HLM (0.5 mg protein/ml) in a final volume of 200 μl. The mixture contained HLM, potassium phosphate buffer (100 mM, pH 7.4), magnesium chloride (3 mM), and the CYP2C9 inhibitor sulfaphenazole (10 μM) or CYP3A4 inhibitor ketocrypton (1 μM). 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 μl 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 μg/mg protein), UGT (0.5 mg/ml), uridine diphosphate glucuronide (UDPGA; 5 mM), magnesium chloride (10 mM), and [1H]Verinurad (10 μM, 2.5 μCi/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 0.5 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 μg/mg protein), UDPGA (5 mM), [1H]Verinurad (10 μM, 2.5 μCi/ml), MgCl2 (10 mM), D-saccharic acid-1,4-lactone (5 mM), and flucanazole (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 μl 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 prefarmed at 37°C for 5 minutes. The incubation was initiated with the addition of M1 or M8 at a final concentration of 10 μM, and 100 μl of internal standard [D6]verinurad at 40 ng/ml. The mixture was incubated at 37°C with the addition of 200 μl 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-α-lysine-coated tissue culture plates at a density of 1.5 × 10⁵ 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, 0.5 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 μl, then 20 μl of 200 μM ³C-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 ³C-uric acid was removed by washing cells three times with Wash Buffer. Cells were lysed by adding 100 μl 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:

\[
\text{percent Inhibition} = 100 - \left( \frac{(100 \text{(Test } - \text{Basal})/[(\text{Control } - \text{Basal})]} \right)
\]

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 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 μM), alamethicin (50 μg/ml), D-saccharic acid-1,4-lactone (3 mM), UDPGA (2 mg/ml), 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.
The terminal half-life of verinurad was 15 hours (Table 2). Geometric mean basis of total radioactivity was 35 hours (Table 2), whereas the plasma biphasic concentration-time profile. Plasma terminal half-life on the postdose (Fig. 3) following oral administration. Verinurad exhibited a administered 10-mg dose of [14C]verinurad was recovered in total over shown graphically in Fig. 2. An average (mean ± S.D.) of 97.1% ± 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%. 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 (±S.D.) of 97.1% ± 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\textsubscript{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\textsubscript{0-\infty} 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 μCi) oral dose of [14C]-verinurad.](image1)

![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 μCi 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.](image2)

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MS/MS Transition (m/z, amu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verinurad</td>
<td>349 → 263</td>
</tr>
<tr>
<td>[D6]Verinurad</td>
<td>355 → 263</td>
</tr>
<tr>
<td>M4</td>
<td>365 → 279</td>
</tr>
<tr>
<td>[D6]M4</td>
<td>371 → 279</td>
</tr>
<tr>
<td>M1</td>
<td>525 → 263</td>
</tr>
<tr>
<td>[D6]M1</td>
<td>531 → 263</td>
</tr>
<tr>
<td>M8</td>
<td>541 → 279</td>
</tr>
<tr>
<td>[D6]M8</td>
<td>547 → 279</td>
</tr>
</tbody>
</table>

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 (±S.D.) of 97.1% ± 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\textsubscript{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\textsubscript{0-\infty} values on the basis of total radioactivity in blood and plasma were

556 and 906 nanogram equivalents · hours/ml, respectively. Blood-to-plasma ratios on the basis of total radioactivity for C\textsubscript{max} and AUC\textsubscript{0-\infty} 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\textsubscript{max} and AUC\textsubscript{0-\infty} were 0.464 and 0.213, respectively, indicating that metabolites were the predominant component in the circulation.

After absorption, verinurad plasma C\textsubscript{max} and AUC\textsubscript{0-\infty} were 160 ng/ml and 193 ng·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\textsubscript{F} of 11.5 l/min, and a high volume of distribution (V\textsubscript{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).
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 $T_{\text{max}}$ 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 $C_{\text{max}}$ and AUC$_{\text{in}}$ were 0.786 and 0.997, respectively. The geometric mean plasma M8-to-verinurad ratios for $C_{\text{max}}$ and AUC$_{\text{in}}$ 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 $C_{\text{max}}$ and AUC$_{\text{in}}$ were 0.550 and 0.321, respectively. Similarly, plasma M8-to-total radioactivity ratios for $C_{\text{max}}$ and AUC$_{\text{in}}$ were 0.350 and 0.313, respectively. The elimination of M1 and M8 were only observed in the urine with high CL$_{\text{0–9.96 hour}}$ 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-glucuronol transferase (UGT) demonstrated that the formation of M1 was mediated by several UG Ts, 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 isomorph 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 IC$_{50}$ was 2.9 ± 0.36 μM (mean ± S.E., n = 10). Minimal inhibition of 14C-uric acid uptake by URAT1 was observed with M8 and did not allow for IC$_{50}$ determination. Because both M1 and M8 were tested at concentrations well above the human $C_{\text{max}}$ 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 [14C]verinurad to eight healthy male

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Matrix</th>
<th>$T_{\text{max}}$</th>
<th>$C_{\text{max}}$</th>
<th>AUC$_{\text{in}}$</th>
<th>CL/F</th>
<th>$V_{\text{d,F}}$</th>
<th>$t_{1/2}$</th>
<th>CL$_{\text{0–9.96 h}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>h</td>
<td>ng/ml</td>
<td>ng·h/ml</td>
<td>l/h</td>
<td>l</td>
<td>h</td>
<td>ml/min</td>
</tr>
<tr>
<td>Verinurad</td>
<td>Plasma</td>
<td>0.50</td>
<td>160</td>
<td>193</td>
<td>51.8</td>
<td>504</td>
<td>14.8</td>
<td>11.5 (7.64–17.3)</td>
</tr>
<tr>
<td>(N = 8)</td>
<td></td>
<td>(0.50–0.75)</td>
<td>(117–219)</td>
<td>(152–246)</td>
<td>(40.7–65.9)</td>
<td>(368–690)</td>
<td>(11.9–18.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>0.50</td>
<td>344</td>
<td>906</td>
<td>—</td>
<td>—</td>
<td>34.7</td>
<td>—</td>
</tr>
<tr>
<td>(N = 8)</td>
<td></td>
<td>(0.50–0.75)</td>
<td>(255–465)</td>
<td>(742–1110)</td>
<td>—</td>
<td>—</td>
<td>(26.9–44.7)</td>
<td>—</td>
</tr>
<tr>
<td>Total radioactivity</td>
<td>Blood-to-plasma ratio</td>
<td>—</td>
<td>0.551</td>
<td>0.614</td>
<td>—</td>
<td>—</td>
<td>(19.9–70.6)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Plasma verinurad-to-total radioactivity</td>
<td>(0.433–0.496)</td>
<td>(0.194–0.234)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

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

$\text{IC}_{50}$ determination. Because both M1 and M8 were tested at concentrations well above the human $C_{\text{max}}$ of 189 and 120 ng/ml (~350 and 220 nM, respectively), contribution to efficacy is low.
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 M1 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
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**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>( T_{max}^{a} )</th>
<th>( C_{max} )</th>
<th>AUC_{tot}</th>
<th>AUC_{u}</th>
<th>t_{1/2}</th>
<th>CL_{10-90%}</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 (N = 8)</td>
<td>0.5 (0.50–0.75)</td>
<td>189 (112–320)</td>
<td>287 (182–452)</td>
<td>290 (185–455)</td>
<td>12.9 (10.2–16.4)</td>
<td>124 (182–276)</td>
</tr>
<tr>
<td>M1-to-verinurad ratio</td>
<td>—</td>
<td>0.786</td>
<td>1.01</td>
<td>0.997</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M1-to-total radioactivity ratio</td>
<td>—</td>
<td>0.661–1.02</td>
<td>0.769–1.34</td>
<td>0.761–1.31</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M8 (N = 8)</td>
<td>0.75 (0.50–0.75)</td>
<td>120 (102–142)</td>
<td>273 (227–329)</td>
<td>283 (235–342)</td>
<td>18.1 (12.4–26.5)</td>
<td>338 (286–399)</td>
</tr>
<tr>
<td>M8-to-verinurad ratio</td>
<td>—</td>
<td>0.485</td>
<td>0.936</td>
<td>0.943</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M8-to-total radioactivity ratio</td>
<td>—</td>
<td>0.381–0.617</td>
<td>0.769–1.14</td>
<td>0.777–1.14</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\[T_{max}\] is expressed as median (range).

---

**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.

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AUC_u, area under the plasma concentration-time curve from zero to infinity; \( t_{1/2} \), half-life;
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 [14C]-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 intraduodenal 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 acylgluronides 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肾-0.06 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.

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 may be 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 Km values (> 10 μM) (Kaivosaari 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.
Conducted experiments: Yang, Shah, Wilson, Ostertag.
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.

References


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Verinurad Human Metabolism