Absorption, Distribution, Metabolism, and Excretion of the Novel Antibacterial Prodrug Tedizolid Phosphate

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ABSTRACT

Tedizolid phosphate is a novel antibacterial prodrug with potent activity against Gram-positive pathogens. In vitro and in vivo studies demonstrated that the prodrug is rapidly converted by nonspecific phosphatases to the biologically active moiety tedizolid. Single oral dose radiolabeled [14C]-tedizolid phosphate kinetic studies in human subjects (100 μCi in 204 mg tedizolid phosphate free acid) confirmed a rapid time to maximum tedizolid concentration (T\text{max}, 1.28 hours), a long terminal half-life (10.6 hours), and a C\text{max} of 1.99 μg/ml. Metabolite analysis of plasma, fecal, and urine samples from rats, dogs, and humans confirmed that tedizolid is the only measurable metabolite in plasma after intravenous (in animals only) or oral administration and that tedizolid sulfate is the major metabolite excreted from the body. Excellent mass balance recovery was achieved and demonstrated that fecal excretion is the predominant (80–90%) route of elimination across species, primarily as tedizolid sulfate. Urine excretion accounted for the balance of drug elimination but contained a broader range of minor metabolites. Glucuronidation products were not detected. Similar results were observed in rats and dogs after both intravenous and oral administration. The tedizolid metabolites showed less potent antibacterial activity than tedizolid. The observations from these studies support once daily dosing of tedizolid phosphate and highlight important metabolism and excretion features that differentiate tedizolid phosphate from linezolid.

Introduction

Tedizolid phosphate (the prodrug form of tedizolid) is a novel oxazolidinone antibiotic that inhibits bacterial protein synthesis (Shaw and Barbachyn, 2011; Kanafani and Corey, 2012) and demonstrates potent in vitro activity against Gram-positive pathogens, including strains resistant to several currently available antibiotics (Shaw and Barbachyn, 2011; Kanafani and Corey, 2012; Locke et al., 2014). In nonclinical studies, tedizolid phosphate demonstrated promising efficacy results across a range of animal infection models (Chan et al., 2009; Louie et al., 2011; Lepak et al., 2012); phase 3 trials showed that administration of intravenous or oral tedizolid phosphate (200 mg once daily) for 6 days achieved noninferior efficacy in acute bacterial skin and skin structure infections compared with 10 days of linezolid therapy (Prokocimer et al., 2011, 2013; Fang et al., 2013).

Oxazolidinones bind to the 50S subunit of bacterial ribosomes, thereby preventing the assembly of the 70S ribosomal complex (Leach et al., 2011; Shaw and Barbachyn, 2011) and effectively inhibiting the initiation phase of translation. Linezolid was the first oxazolidinone antibiotic approved by the U.S. Food and Drug Administration to treat serious Gram-positive infections (Leach et al., 2011), and it established a background understanding of the pharmacokinetic (PK), metabolic, and allometric attributes of an antibacterial oxazolidinone (Wynalda et al., 2000; Slatter et al., 2001; Bhamidipati et al., 2004). However, growing concern over linezolid resistance associated with point mutations and horizontally transferred resistance genes (Locke et al., 2014) has spurred interest in the development of new oxazolidinone molecules. Although numerous oxazolidinone analogs with modifications to the A-, C-, and D-rings have been screened for activity, only five molecules have moved to mid- or late-stage development, including tedizolid (Leach et al., 2011; Shaw and Barbachyn, 2011; Locke et al., 2014). To enhance the bioavailability of tedizolid, a series of prodrug formulations were evaluated, and the phosphate analog was selected for further clinical development, based on its enhanced water solubility, its stability across a pH range of 3 to 8, and its rapid hydrolysis by endogenous phosphatases. The present studies explored the relative absorption, distribution, metabolism, and excretion of tedizolid phosphate in mice, rats, dogs, and humans.

Materials and Methods

Tedizolid phosphate (disodium salt for animal studies; free acid for human studies) and tedizolid were synthesized by Albany Molecular Research (Albany, NY). Radiolabeled tedizolid phosphate (14C on the lone carbon in the tetrazole ring) was obtained from ChemDepo (Camarillo, CA) for the animal studies and from Kalexsyn (Kalamazoo, MI) for the human clinical study. For studies in animal or human subjects, radiolabeled [14C]-tedizolid phosphate was synthesized with specific activities of 0.107 or 0.122 mCi/mg, respectively.

This study was sponsored by Cubist.

Employees of the study sponsor were involved in study design, data collection, data analysis, interpretation of the results, and review/writing of the manuscript. All authors had full access to the data. The authors had final responsibility for the decision to submit for publication. V. Ong, S. Flanagan, E. Fang, H.J. Dreskin, J.B. Locke, K. Bartizal, and P. Prokocimer are employees of Cubist. Medical writing support was provided by Bill Jacobs (Strategic HealthCom, Somerville, NJ), funded by Cubist.

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ABBREVIATIONS: AUC, area under the curve; AUC\text{0-last}, area under the curve from time zero to time of last measurable concentration; C\text{max}, maximum observed concentration; HPLC, high-performance liquid chromatography; MIC, minimum inhibitory concentration; MS/MS, tandem mass spectrometry or mass spectrometry/mass spectrometry; PK, pharmacokinetic; SULTs, sulfotransferases.
Tedizolid Analysis from Blood/Plasma and Tissue Samples

Extracts of in vitro buffers, blood/plasma samples, and organs from study animals were collected at predesignated time points (see below) and then processed, centrifuged, and frozen until analysis was conducted. Processed samples were analyzed by high-performance liquid chromatography (HPLC) with tedizolid identified by retention time and quantified by UV absorption, scintillation counting, or mass spectrometry (as described below).

In Vitro Metabolism and Metabolic Profiling

Stability of Tedizolid Phosphate in Plasma. Blood samples from rats, dogs, and humans were collected (used either heparin or EDTA as anticoagulant) and corresponding plasma was harvested. Fresh plasma was divided into 200-μl aliquots to which tedizolid phosphate was added to achieve a concentration of 2 μg/ml. Plasma samples were incubated at 37°C for 0, 5, 10, 15, 20, and 30 minutes and 1 and 2 hours. A 200-μl aliquot of 1 M hydrochloric acid was added to quench the reaction. Conversion of the prodrug to tedizolid was assessed by HPLC with tandem mass spectrometry (MS/MS) detection.

Protein Binding of Tedizolid. Samples of rat, mouse, dog, and human plasma were collected (prepared with either heparin or EDTA as the anticoagulant) and incubated with 1 or 10 μg/ml (final incubation concentrations achieved by spiking 5 μl of 50% acetonitrile stock solution into 0.5 ml plasma) tedizolid or tedizolid phosphate at 37°C for 30 minutes and then separated by ultrafiltration through a Ultrafree-MC (EMD Millipore, Billerica, MA; 30,000 molecular weight cut off) low-binding cellulose filter at 37°C. The ultrafiltrate was extracted, and tedizolid or tedizolid phosphate was quantified by reverse-phase HPLC with UV detection.

Liver Metabolism. Animal and human liver microsomes (0.5 mg/ml) and S9 (0.75 mg/ml) fractions (pooled male; BD Biosciences, San Jose, CA) were incubated with 10 or 20 μM tedizolid phosphate with and without reduced nicotinamide adenine dinucleotide phosphate (NADPH) in a final volume of 400 μl containing 0.5 M potassium phosphate (100 mM, pH 7.4) at 37°C to support available cytochrome P450 enzyme activity. To terminate the reaction (samples collected at 30, 60, 90, and 120 minutes), 50 μl of the mixture was added to a clean tube containing ethyl acetate (0.6 ml), 1 M hydrochloric acid (50 μl), and sodium citrate buffer (pH 1.8, 0.5 ml). Conversion of tedizolid phosphate to tedizolid was monitored by HPLC with mass spectrometry detection of tedizolid phosphate and tedizolid levels over time.

Distribution of Tedizolid or Linezolid in Plasma and Whole Organs

In an initial study to compare the tissue distribution of tedizolid or linezolid, nonlabeled tedizolid phosphate (13.3 mg/kg; molar equivalent to 10 mg/kg tedizolid or linezolid (10 mg/kg) was administered intravenously to male Sprague-Dawley rats at a dose volume of 1 ml/kg. Animals were fasted overnight and split into two groups of 3 animals each. One group received a single intravenous dose by tail vein injection of tedizolid phosphate (13.3 mg/ml, dissolved in distilled water), and the second group received linezolid [10 mg/ml, dissolved in dimethylacetamide/polyethylene glycol-400/distilled water (2.5/5/2.5, vol/vol/vol) at 10 mg/kg]. Blood samples were collected through 6 hours (approximately 3 times the terminal half-life), at which time animals were killed and organs were harvested to determine tissue tedizolid or linezolid levels by HPLC with UV detection.

Animal Absorption, Distribution, Metabolism, and Excretion Studies

To assess plasma pharmacokinetics of tedizolid phosphate and tedizolid, a single dose of tedizolid phosphate dissolved in distilled water was administered to male rats via intravenous injection into the jugular vein over 1 minute at doses of 5, 10, or 20 mg/kg (n = 7, 7, and 9, respectively) at a dose volume of 2 ml/kg. Tedizolid phosphate dissolved in distilled water was also administered to male rats via oral gavage at doses of 20, 50, or 100 mg/kg (n = 11, 9, and 9, respectively) at a dose volume of 3 ml/kg. After intravenous dosing, samples were collected at 0 (minute) (control), 1 (at end of infusion), 5, 15, 30, and 45 minutes and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours. After oral dosing, samples were collected at 0, 15, and 30 minutes and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours. The plasma was then harvested to determine the concentrations of tedizolid phosphate or tedizolid by HPLC with UV detection. The pharmacokinetics of tedizolid were also determined in male and female Sprague-Dawley rats after administration of a single 20 mg/kg dose of tedizolid phosphate either intravenously or orally and in Beagle dogs after the administration of a single 10 mg/kg dose of tedizolid phosphate either intravenously or orally (in a gelatin capsule). For the rat sex comparison study, tedizolid phosphate dissolved in distilled water was administered as a 20 mg/kg dose to male and female rats (N = 6–8/sex) via intravenous injection (2 ml/kg dose volume as 10 mg/ml solution) into the jugular vein over 1 minute or via oral gavage (3 ml/kg dose volume as 6.67 mg/ml solution). After intravenous dosing, samples were collected at 0 minute (control), 1 (at end of infusion), 5, 15, 30, and 45 minutes and at 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours. After oral dosing, samples were collected at 0, 15, and 30 minutes and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours. For the dog sex comparison study, tedizolid phosphate dissolved in distilled water was administered as a 20 mg/kg dose to male and female dogs (N = 3/sex) via intravenous injection (0.5 ml/kg dose volume as 20 mg/ml solution) into the cephalic vein over 1 minute or via oral gavage as a gelatin capsule coadministered with 7.5 ml/kg of water. After dose administration, plasma samples were collected at 0, 1 (intravenous only), 5, 15, and 30 minutes and 1, 2, 4, 6, and 10 hours. To ascertain the metabolism and excretion of tedizolid phosphate in male and female rats and dogs, a single dose of 14C-labeled tedizolid phosphate was administered as an intravenous bolus (10 mg/kg) or orally (25 mg/kg by gavage. Separate groups of rats (Sprague-Dawley, 7/sex/route) and Beagle dogs (5/sex/route) were used in these studies. Doses were adjusted to target so that the radioactivity administered was 100 μCi/kg in rat and 25 μCi/kg in dog. For rats, dose-formulation concentrations were 5 mg/ml for intravenous dosing (in a 2 ml/kg dose volume) and 2.5 mg/ml for oral dosing (in 10 ml/kg dose volume). For dogs, dose formulation concentrations were 10 mg/ml for intravenous dosing (in 1 ml/kg dose volume) and 5 mg/ml for oral dosing (in 5 ml/kg dose volume). All dosing solutions were prepared in 0.9% (w/v) sodium chloride. After dose administration, the radioactivity in whole blood and plasma was determined at 10 minutes (dog only) and at 0.5 (dog only), 1, 2, 4, 8, 24, 72, and 168 (rat only) hours postdosing. Urine was collected over the following intervals: 0 to 6, 6 to 12, and 12 to 24 hours post dosing and then every 24 hours through 168 hours (rat) and 72 (dog) hours post dosing. Feces were collected over the following intervals: 0 to 12 and 12 to 24 hours post dosing and then every 24 hours through 168 (rat) and 72 (dog) hours post dosing. Whole blood, plasma, urine, and fecal radioactivity levels were determined by liquid scintillation counting (LSC). Plasma and urine samples were mixed with an Ultima Gold (PerkinElmer, Waltham, MA) scintillation cocktail for direct analysis by liquid scintillation counting, whereas blood and fecal samples were combusted in a Harvey Biologic Materials Oxidizer Model MX500 (R. J. Harvey Instrument Corp., Hilldale, NJ) and the resultant 14CO2 was trapped in a mixture of aqueous mounting medium (PermaFluor; PerkinElmer) and radioactive dioxide absorber (Carbo-Sorb; PerkinElmer) before radioanalysis. Radioactivity was determined using a Beckman Model LS 6000TA or LS 6500 liquid scintillation spectrophotometer (Beckman Coulter, Brea, CA).

Absorption, Metabolism, and Excretion of Tedizolid in Humans

A phase 1, open-label, nonrandomized, single-dose, single-center, mass balance study was conducted to investigate the absorption, metabolism, and excretion of tedizolid phosphate after oral administration of 14C-radiolabeled tedizolid phosphate (100 μCi in 204 mg tedizolid phosphate). The study was conducted according to the principles of good clinical practice and applicable regulatory requirements and was consistent with the Declaration of Helsinki. Healthy male volunteers between 18 and 50 years of age were screened for overall health (physical examination, clinical laboratory tests, ECG evaluation) and absence of medications or use of herbal or dietary products that could interfere with metabolic processes. Screening occurred within 28 days of study entry. Subjects were admitted to the clinical research unit 1 day before study entry (day −1) and fasted for 8 hours before the administration of study drug. Six enrolled subjects completed the study; there were no early withdrawals.

On the morning of the second day (day 1), 204 mg of radiolabeled tedizolid phosphate (100 μCi of 14C-tedizolid phosphate) was administered orally in a 240-ml solution, followed by collection of plasma, urine, and fecal samples at predetermined time intervals between 0.5 and 312 hours post dose. Blood samples for determination of total radioactivity in whole blood and plasma were collected at the following time points: predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, and 312 hours post dose. Blood samples for determination of plasma concentrations...
were collected at the following timepoints: predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours postdose. Blood samples for metabolite profiling in plasma were collected at the following timepoints: predose and 2, 4, 6, 12, 24, 48, 72, 96, and 120 hours postdose. Urine was collected at the following intervals: predose and 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 192, 192 to 216, 216 to 240, 240 to 264, 264 to 288, and 288 to 312 hours postdose. Feces were collected at the following intervals: predose and 0 to 24, 24 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 192, 192 to 216, 216 to 240, 240 to 264, 264 to 288, and 288 to 312 hours postdose. Although they were in the study center, subjects received a standard diet according to a schedule that did not interfere with the sampling schedule. Subjects were evaluated by physical examination, 12-lead ECG, determination of vital signs, and laboratory values. All subject-reported and physician-noted adverse events were recorded. Subjects were discharged from the study center when plasma radioactivity was below the lower limit of detection, ≥90% of the administered dose had been recovered, and fecal/urine radiotracer excretion was ≤1% of the administered dose for 2 consecutive days.

After sample collection and initial processing, blood/plasma samples were stored at −70°C, and urine and feces samples were stored at −20°C until analysis. Plasma and urine samples were centrifuged and analyzed directly by liquid scintillation counting after addition of a scintillation cocktail (Ultima Gold; PerkinElmer). Blood and homogenized fecal samples were combusted in a Packard Model 307 Sample Oxidizer (Packard Instruments, Downers Grove, IL), and the resultant 14CO2 was trapped in a mixture of PermaFluor and Carbotrap (PerkinElmer). Blood and urine samples were centrifuged and analyzed directly by liquid scintillation counting. Plasma and urine samples were centrifuged and analyzed directly by liquid scintillation counting. For plasma or blood concentration values that were below the limit of detection, 0.0125 x 10−6 counts per minute was used for plasma or blood concentration values that were below the limit of detection. For plasma, the volume of each sample was included. Each pooled plasma and fecal sample was extracted with acetonitrile (sample: acetonitrile, 1:5, v/v), vortex mixed, sonicated, and centrifuged, followed by removal of the supernatant. The supernatant was evaporated to near dryness and reconstituted for subsequent metabolite profiling. Urine samples were pooled by subject to generate single 0- to 12-hour and 12- to 48-hour pooled samples, including 0.275 to 0.55% (equivalent percent by interval) of each sample, and analyzed directly. Metabolites were characterized and identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and were quantified by HPLC with radiometric detection. Quantitative HPLC was performed with an HP 1100 series system (Hewlett-Packard, Palo Alto, CA) with a 4.6 × 150 mm Xterra RP 18 (3.5 μm particle size) column (Waters, Milford, MA) with a C18 guard column (3 × 4 mm; Phenomenex, Torrance, CA). The parent compound and the metabolites were eluted using a binary solvent gradient, from 99% 25 mM ammonium acetate (pH 8.5) and 1% acetonitrile to 1% ammonium acetate and 99% acetonitrile over 37 minutes, at a flow rate of 1 ml/min. For metabolite quantification from plasma, urine, or fecal samples, the HPLC eluent was fractionated at 10-second intervals into 96-well titer plates and were analyzed for radioactivity using a TopCount scintillation counter (PerkinElmer). Metabolites were identified by retention time and quantified based on the radioactivity profile for each peak.

For mass spectrometry analysis, a Prominence HPLC system (Shimadzu, Kyoto, Japan) was used with the same column and binary gradient described for quantitative HPLC. The eluent was split with 25% directed to an Orbitrap XL mass spectrometer (Thermo Fisher Scientific) with an electrospray interface. Capillary temperature, gas flow, source/capillary voltages, and tube lens voltage were optimized for parent compound and metabolite characterization. Data-dependent acquisition identified compounds in a full-scan event cycle and two high-resolution scans. Metabolites were identified by HPLC retention time and subsequent mass spectrometry analysis.

**Antibacterial Activity of Tedizolid Metabolites**

Minimum inhibitory concentrations (MIC) were determined by broth microdilution according to Clinical and Laboratory Standards Institute-approved methods (CLSI, 2012). Staphylococci (Staphylococcus aureus strains ATCC 13709, ATCC 33591, and RN4220; Staphylococcus epidermidis ATCC 12228) and enterococci (Enterococcus faecalis ATCC 29212; Enterococcus faecium ATCC 19434 and ATCC 700221) were incubated in Mueller-Hinton agar or cation-adjusted Mueller-Hinton broth. Streptococci (Streptococcus pneumoniae ATCC 49619 and ATCC 51916; Streptococcus pyogenes ATCC 19615) were cultured in tryptic soy agar with 5% sheep blood or Mueller-Hinton broth with 3% laked horse blood. Stock solutions of tedizolid and the desmethyl-, sulfate-, and carboxy-metabolites were prepared in 100% dimethylsulfoxide and serially diluted in twofold steps. Stock metabolite solutions (2 μl at 50× final assay concentration) were added to 98 μl media for a final metabolite concentration range of 0.00625 to 128 μg/ml. Similar dilutions of stock solutions of tedizolid were prepared for a concentration range of 0.0156 to 32 μg/ml. Plates were incubated overnight at 37°C and for an additional hour after addition of 10 μl AlamarBlue (Baker and Tenover, 1996). MIC values were determined as the concentration with no visible color change.

**Data Analysis**

For studies in animals and human subjects, continuous data are summarized using descriptive statistics, and categorical data are expressed as counts or percentages. Nonparamatral pharmacokinetic analysis was performed, as appropriate, using WinNonlin version 5.2 (Pharsight Corporation, Cary, NC) computational software. Concentration values were not rounded prior to the calculation of PK parameters; the concentrations were used as supplied by the analytical laboratory for bioanalysis. Plasma or blood concentration values that were below lower limits of detection (5 ng/ml) were set to zero for the purposes of PK analysis. For human subjects, safety was assessed using summaries of adverse events, clinical tests results, laboratory findings, vital signs, physical examinations, and ECGs by treatment group or sequence. Main effects were determined using an analysis of variance, with post hoc Dunnett’s testing to identify significant differences from control values. Standard descriptive statistics were calculated for all PK parameters.
Results

Tedizolid In Vitro Metabolism. The prodrug tedizolid phosphate was found to be stable in EDTA-treated plasma but not in heparin-treated plasma. When incubated in heparinized plasma samples from mice, rats, dogs, or humans, tedizolid phosphate was converted to tedizolid, although the conversion rate was noticeably slower in rats (Table 1). Tedizolid phosphate protein binding in EDTA-treated plasma samples was 97.2% for rat, 74.8% for mouse, 86.6% for human, and 85.1% for dog samples. Mean plasma protein binding for tedizolid in EDTA-treated plasma samples was 97.7% for rat, 92.6% for mouse, 84.6% for human, and 78% for dog samples. Protein binding for tedizolid was similar when EDTA or heparin was used as the anticoagulant.

When incubated with liver microsome preparations, tedizolid phosphate was stable over 2 hours in the absence (99, 86, 111, and 91% recovered for mice, rats, dogs, and humans, respectively) or presence (107, 88, 93, and 103% recovered, respectively) of NADPH. This observation differs from the metabolism of linezolid, which is oxidized through an NADPH-dependent, noncytochrome P450-mediated process in rat liver microsomes (Slatter et al., 2002; Leach et al., 2011). When incubated in an S9 fraction preparation from rats, 62% of the starting tedizolid phosphate remained intact after 2 hours in the absence of NADPH and only 49% remained in the presence of NADPH, suggesting that the S9 fraction contains the phosphatases necessary for conversion. Indeed, tedizolid phosphate conversion to tedizolid was not observed when a cocktail of phosphatase inhibitors (imidazole, sodium fluoride, sodium molybdate, sodium orthovanadate, and sodium tartrate dehydrate) was added to the reaction mixture (data not shown).

Distribution of Tedizolid or Linezolid in Plasma and Whole Organs. In rats, tedizolid distribution was highest in the gastrointestinal tract, liver, kidney, heart, and lungs, with lower levels in other organs examined (Fig. 1). Linezolid distribution was consistent with previous reports (Slatter et al., 2002) and generally paralleled the distribution of tedizolid. The clinical use of linezolid has been associated with increased risk for thrombocytopenia and central nervous system interactions with selective serotonin reuptake inhibitors (Leach et al., 2011; Shaw and Barbachyn, 2011). In light of these characteristics, it was interesting to note that steady-state tissue:plasma ratios for tedizolid were lower in bone (tissue:plasma ratio of 0.2 and 0.4 for tedizolid and linezolid, respectively) and brain (tissue:plasma ratio of 0.06 and 0.3 for tedizolid and linezolid, respectively). In dogs, tissue:plasma area under the curve from time zero to time of last measurable concentration (AUC0-last) was highest in the intestines (large, 21; small, 10), liver (11), and kidneys (5) and lowest in bone, brain, and eye (<0.25). When administered intravenously, the tissue:plasma AUC0-last was highest in the intestines (large, 5; small, 10), liver (11), and kidney (12) and lowest in bone, brain, and eye (<0.25).

Animal Pharmacokinetic and Absorption, Distribution, Metabolism, and Excretion Studies. After a single dose in male rats, plasma tedizolid phosphate levels fell rapidly after intravenous administration and were significantly lower when the prodrug was administered orally, consistent with rapid prodrug hydrolysis to tedizolid during and/or immediately after absorption. Tedizolid rapidly appeared in plasma (Tmax at 0.13 to 0.2 hours after administration), with a subsequent multiexponential decline over time (Fig. 2). In rats, the overall blood/plasma AUC0-last was 0.6, suggesting that 14C derived radioactivity did not partition well into red blood cells, likely because of tedizolid’s high protein binding.

In subsequent studies with male and female rats treated with tedizolid phosphate, the AUC was >3-fold higher in female rats and was associated with a longer terminal half-life (Table 2), whereas Cmax was only modestly increased (~37%) on intravenous administration with no change observed on oral administration. Within each sex, kinetic profiles were similar when tedizolid phosphate was administered intravenously or orally, aside from reduced Cmax with oral administration. Pharmacokinetics in male Beagle dogs were also evaluated after administration of a single 10 mg/kg oral dose of tedizolid phosphate (Table 2). Overall bioavailability of tedizolid after oral administration of tedizolid phosphate ranged from 73 to 87% in rats and 63% in dogs.

For metabolism and mass balance excretion studies, recovery of total radioactivity after administration of 14C-labeled tedizolid phosphate was >95% from rats and dogs (extraction recovery for all sample types ranged from 96.4 to 111%). Regardless of the route of tedizolid phosphate administration (intravenous or oral), fecal excretion (80–90% of dose) was the primary route of elimination in both species, independent of sex.

![Fig. 1. Mean tissue-to-plasma concentration ratios at 6 hours after a single intravenous dose of tedizolid phosphate (13.3 mg/kg, molar equivalent to 10 mg/kg tedizolid) or linezolid (10 mg/kg) in rats.](image-url)
Urinary excretion accounted for approximately 10% of the dose. In rats, only one metabolite (tedizolid) was evident in plasma samples, whereas two major metabolites (tedizolid sulfate and tedizolid) were detected in fecal samples in the first 24 hours after intravenous or oral administration (Table 3). See Supplemental Fig. 1 for the plasma radioactivity concentration-time profile and Supplemental Table 1 and Supplemental Fig. 2 for a detailed summary of the metabolite identification data in the rat.

Urinary excretion of tedizolid phosphate metabolites was quantitatively less than the fecal route, but a more complex metabolite profile was observed in urine samples. Notably, seven urinary metabolites were identified during the first 6 hours after administration in rats (Table 4). Regardless of the route of administration, the sulfate metabolite was prominent at 6 hours and was the dominant metabolite excreted over 24 hours. Interestingly, the second highest metabolite in the urine of male rats was desmethyl sulfate tedizolid, whereas the desmethyl metabolite was the second highest metabolite in the urine of female rats. Radiolabeled tedizolid phosphate was not detected in fecal, urine, or plasma samples from rats.

Mass balance radiolabel studies in dogs largely confirmed the findings from rats. After oral (10 mg/kg target dose) or intravenous (25 mg/kg target dose) administration of tedizolid phosphate, approximately 100% of the total dose was recovered, with the majority in feces (~90% of the administered dose) and the rest in urine (~10% of the administered dose) over 72 hours. Analysis of fecal samples confirmed that the majority of radioactivity was attributed to tedizolid sulfate, with minor contributions from desmethyl tedizolid and tedizolid (Table 3). Urinary excretion (~10% of administered dose) was predominantly tedizolid sulfate or desmethyl tedizolid (Table 4). Extraction recovery for all sample types ranged from 86 to 115%. In contrast to the complex sex-dependent pattern observed in rats, additional metabolites were not readily detected in the urine of male or female dogs. Tedizolid metabolites were also not detected in plasma samples from dogs after intravenous or oral administration. See Supplemental Fig. 3 for the plasma radioactivity concentration-time profile and Supplemental Table 2 and Supplemental Fig. 4 for a detailed summary of the metabolite identification data in the dog.

**Human Pharmacokinetics and AME.** When a single 204-mg oral dose of 14C-radiolabeled tedizolid phosphate was administered to humans, the prodrug also underwent rapid hydrolysis to tedizolid. The majority of the recovered radioactivity in plasma (94.5–98.2%) was tedizolid, and the ratio of whole blood to plasma total radioactivity was 0.9, roughly consistent with preclinical evidence that the majority of radioactivity is moderately associated with red blood cells. After oral administration, plasma Cmax and AUC for tedizolid was approximately 95% of total radioactivity. Time to maximum concentration was slightly longer for total radioactivity (1.5 hours) compared with tedizolid (1.28 hours), whereas the elimination half-life of total radioactivity (11.5 hours) was slightly longer than the half-life for tedizolid (10.6 hours) (Table 5). Tedizolid volume of distribution was estimated to be 101 l.

Radioactivity recovery was 87.6% complete at 96 hours postdose and 99.5% complete after 288 hours, at which point 81.5% was recovered in feces and only 18% was recovered in urine (Fig. 3). In contrast to plasma sample radioactivity that was nearly all tedizolid, only 3% of the administered dose was recovered in feces and urine combined as tedizolid (Table 6). Good extraction/reconstitution recoveries were obtained for plasma, urine, and fecal samples and

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**TABLE 2**

Single-dose tedizolid pharmacokinetic parameters across species and sex (rat) after administration of tedizolid phosphate

<table>
<thead>
<tr>
<th></th>
<th>Sprague-Dawley Rats</th>
<th>Beagle Dogs</th>
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<tbody>
<tr>
<td></td>
<td>Male 20 mg/kg*a</td>
<td>Female 20 mg/kg*</td>
</tr>
<tr>
<td>Oral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>14.6</td>
<td>14.6</td>
</tr>
<tr>
<td>Tmax (hour)</td>
<td>0.50</td>
<td>0.96</td>
</tr>
<tr>
<td>T1/2 (hour)</td>
<td>3.7</td>
<td>7.0*</td>
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<tr>
<td>AUC0-Last</td>
<td>45.2</td>
<td>144*</td>
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<tr>
<td>F</td>
<td>76%</td>
<td>71%</td>
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|                        |                     |              |              |
| Intravenous            |                     |              |              |
| Cmax (µg/ml)           | 42.4                | 58.1***      | 5.37         |
| Tmax (hour)            | 0.06                | 0.08         | 0.08         |
| T1/2 (hour)            | 1.6                 | 4.1***       | 0.58         |
| AUC0-Last              | 59.3                | 202***       | 4.4          |

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*Cmax, maximum drug concentration; Tmax, time to maximum drug concentration; T1/2, drug half-life; AUC0-Last, area under the curve from time zero to last measurable sample; F, percentage absolute bioavailability, calculated as [(AUC oral × dose intravenous)/(AUC intravenous × dose oral)] × 100.

*aAdministered dose of tedizolid phosphate. Oral tedizolid phosphate was administered to rats by gavage and to dogs in gel capsules.

*p < 0.05 versus males; **p < 0.001 versus males; ***p < 0.01 versus males.
ranged from 86.6 to 96.1%, 91.0 to 100%, and 88.3 to 100%, respectively.

Poole fecal samples showed one major (tedizolid sulfate) and two minor (tedizolid and carboxy tedizolid) metabolites (Table 6). Poole urine samples showed one major (tedizolid sulfate) and three minor (carboxy tedizolid, tedizolid, and desmethyl tedizolid) metabolites (Table 6). See Supplemental Fig. 5 for the plasma radioactivity concentration-time profile and Supplemental Table 3 and Supplemental Fig. 6 for a detailed summary of the metabolite identification data in human. Four trace metabolites (desmethyl tedizolid sulfate, ring-opened tedizolid metabolite, and two unidentified molecules) were also detected in urine, but they represented <1% of the administered dose each. Comparison of the urine metabolite composition from samples collected between 0 to 12 hours and 12 to 24 hours after tedizolid phosphate administration revealed a similar metabolite profile between the two collection periods, except for tedizolid levels, which were higher in the first 12-hour sample.

Overall, the sulfated analog was the primary metabolite excreted from the body, accounting for elimination of 69% (56.81–79.49%) of the oral dose in feces and 10% (7.17–14.2%) of the administered dose in urine. Demethylation and nonring opening oxidation were minor biotransformation processes. A proposed pathway for tedizolid metabolism is shown in Fig. 4.

**Antibacterial Activity of Tedizolid Metabolites.** Initial structural consideration suggested that the desmethyl, but not the sulfate, metabolite might possess antibacterial activity. Both the desmethyl and sulfate metabolites demonstrated lower activity than tedizolid against all test strains (Table 7). Further evaluation suggested that the activity associated with the sulfate metabolite may be related to degradation of the test sample to tedizolid. The carboxy metabolite consistently demonstrated MIC values >128 μg/ml (data not shown). In contrast, the potent activity of tedizolid was consistent with previous studies conducted in numerous Gram-positive pathogens, including strains resistant to linezolid and vancomycin (Locke et al., 2014).

**Discussion**

Tedizolid phosphate is a novel prodrug antibiotic under investigation for infections due to Gram-positive pathogens, including acute bacterial skin and skin structure infections and nosocomial pneumonia. In a series of in vitro studies, the metabolism of tedizolid phosphate was shown to involve rapid hydrolysis of the prodrug to the free active moiety tedizolid in plasma and in S9 cellular fractions, but not in the microsomal fraction. Thus, the rapid conversion of tedizolid phosphate was consistent with the activity of nonspecific phosphatases, as confirmed by inhibitor studies. In vitro studies demonstrated high tedizolid protein binding to animal and human plasma, compared with the 31% binding of linezolid (Stalker and Jungbluth, 2003). Therefore, protein binding may serve as a drug reservoir for extended plasma half-life. Tissue distribution studies demonstrated comparable tedizolid and linezolid levels for most organs examined. Additional testing suggested lower tedizolid concentrations in bone and brain (relative to plasma) compared with linezolid. Tedizolid distribution in bone was of particular interest, because hematologic abnormalities are a noted adverse effect associated with linezolid use (Stalker and Jungbluth, 2003). Similarly, the lower tissue-to-plasma concentration in brain is consistent with the lower risk.
for central nervous system-related monoamine oxidase interactions previously observed in animal models that compared tedizolid and linezolid (Flanagan et al., 2013).

Tedizolid was the only metabolite consistently observed in plasma samples after intravenous or oral administration of tedizolid phosphate. The plasma PK profile for tedizolid in animals and humans is consistent with rapid conversion of the prodrug to microbiologically active tedizolid, with slower plasma clearance of tedizolid. Although the $T_{\text{max}}$ for tedizolid was similar to values seen with linezolid (Stalker and Jungbluth, 2003), the terminal half-life of tedizolid (10.6 hours) was approximately double the lower 5.4-hour half-life of oral linezolid (Stalker and Jungbluth, 2003). This longer half-life supports the use of once daily tedizolid dosing in clinical practice. The volume of distribution for tedizolid (101 l) was also larger than the steady-state estimated volume of 40 to 50 l reported for linezolid (Stalker and Jungbluth, 2003).

Mass balance studies in rats and humans demonstrated that the majority of administered tedizolid phosphate was eliminated as a sulfate conjugate of tedizolid, primarily through presumed biliary excretion into feces. Cleavage of the prodrug phosphate group leaves an alcohol side group that appears to be a preferred target for sulfate conjugation. Sulfonation and glucuronidation are important phase 2 metabolic processes (Jakoby and Ziegler et al., 1990; Jia and Liu et al., 2007; Wu et al., 2011) mediated by stereospecific transferase enzymes (Wu et al., 2011; Tang et al., 2012). Sulfonation is a major conjugation pathway for phenols, alcohols, and amine-based structures, catalyzed by a family of sulfotransferases (SULTs) located in the cytosol (for metabolism of xenobiotics and small endogenous substrates) or bound to the Golgi apparatus (responsible for sulfonation of peptides, proteins, lipids, and glycosaminoglycans) (Gamage et al., 2006; Lindsay et al., 2008). Das and co-workers (2012) recently showed that subcellular tedizolid distribution is consistent with cytosolic drug accumulation, suggesting that cytosolic SULTs are most likely involved in tedizolid metabolism. It is interesting that glucuronidation products of tedizolid were not detected, despite similar targeting of alcohol groups by UDP glucuronosyltransferases and SULTs and the proclivity for mixed conjugation reactions when reactive groups are available (Gamage et al., 2006; Jia and Liu et al., 2007; Wu et al., 2011).

A sex-related difference in urinary excretion of the minor metabolites desmethyl (female predominant) and desmethyl-sulfate (male predominant) was noted in rat studies and may yield insight into the cellular processing or SULT isoforms involved with tedizolid metabolism (Jia and Liu et al., 2007). Whether this difference contributes to the longer

### TABLE 5

Plasma pharmacokinetics of tedizolid and total radioactivity after administration of tedizolid phosphate in humans

<table>
<thead>
<tr>
<th></th>
<th>Plasma Tedizolid</th>
<th>Plasma Total Radioactivity (tedizolid equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/ml or µg-Eq/ml)</td>
<td>1.99 (0.33)</td>
<td>2.56 (0.47)</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hour)</td>
<td>1.28</td>
<td>1.5</td>
</tr>
<tr>
<td>$T_{1/2}$ (hour)</td>
<td>10.6 (1.79)</td>
<td>11.5 (1.85)</td>
</tr>
<tr>
<td>$AUC_{0-t}$ (µg·h/ml or µg-Eq·h/ml)</td>
<td>24.7 (4.96)</td>
<td>25.8 (5.45)</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (µg·h/ml or µg-Eq·h/ml)</td>
<td>25.8 (5.46)</td>
<td>27.3 (5.77)</td>
</tr>
<tr>
<td>$Vz/F$ (l)</td>
<td>101 (15.1)</td>
<td>NA</td>
</tr>
</tbody>
</table>

AUC, area under the curve, for a specific time interval; $C_{\text{max}}$, maximum observed plasma concentration; NA, not applicable; $T_{\text{max}}$, time to maximum plasma concentration; $T_{1/2}$, apparent terminal elimination half-life; $Vz/F$, volume of distribution.

Fig. 3. Mean cumulative percentage of radioactive dose was recovered in urine and feces after single 204-mg (100-µCi) oral $^{14}$C-tedizolid phosphate to healthy male subjects. Error bars represent standard deviation.
Interestingly, the sex differences were not noted in dog studies, suggesting that unique differences in SULT expression or isoform availability in rodents may account for the sex differences. Variations in SULT isoform, copy number, and polymorphisms have also been described across ethnic backgrounds in humans (Carlini et al., 2001; Nagar et al., 2006; Hildebrandt et al., 2007; Hebbring et al., 2008).

Tedizolid phosphate was stable in microsome preparations, further distinguishing tedizolid metabolism from that of linezolid, which is primarily metabolized through microsome-mediated morpholine ring oxidation (that does not involve the P450 system, flavine monoxygenase, or monoamine oxidase activity) with the formation of two major metabolites of linezolid (PNU-142300 and PNU-142586), which can also be detected in human plasma (equivalent to 20–30% of the total AUC) after linezolid administration (Wynalda et al., 2000; Slatter et al., 2001). Minor oxidative opening of the oxazolidinone ring has also been observed when rivaroxaban was incubated with liver microsomes from rats, dogs, and humans (Lang et al., 2009), but the metabolite has not been detected in plasma or excreta in human mass balance studies (Weinz et al., 2009).

The major route of tedizolid excretion is fecal, mainly as the sulfate metabolite, unlike the predominantly renal elimination of linezolid (35% of an administered linezolid dose is excreted in urine as linezolid, 40% as PNU-142586, and 10% as PNU-142300) (Stalker and Jungbluth, 2003). Although the full physiologic impact of linezolid metabolites is unknown, it is interesting to note that mild to severe renal impairment is associated with accumulation of these metabolites, resulting in 6.5- to 7.4-fold increases in metabolite AUC from patients with normal renal function to those with severe renal impairment (Stalker and Jungbluth, 2003); the risk for adverse hematologic effects of linezolid also increases with declining renal function (Takahashi et al., 2011). Nonlinear kinetics of linezolid with time may be related to decreased linezolid metabolism by an energy-dependent pathway caused by linezolid’s effects on mitochondria (Plock et al., 2007).

To assess the potential impact of hepatic and renal impairment on tedizolid pharmacokinetics, two phase 1 trials were undertaken in human subjects with mild or severe hepatic impairment or severe renal impairment, including subjects on dialysis (Flanagan et al., 2012a,b).

In subjects with hepatic impairment, there was no significant change in tedizolid $C_{\text{max}}$ after single-dose administration of 200 mg tedizolid phosphate. The AUC was 39% greater in severe and 22% greater in moderate hepatic impairment subjects compared with a matched control population. For subjects with severe renal impairment, $C_{\text{max}}$ and AUC were unaltered compared with matched control populations after intravenous administration of a single 200-mg dose of tedizolid phosphate, and hemodialysis does not extract appreciable amounts of tedizolid from blood.

The major metabolites of tedizolid are not present in the circulation and possess less potent antibacterial activity, providing support that it is primarily tedizolid exposures that will determine efficacy against serious infections caused by Gram-positive bacteria. In addition, given that unchanged tedizolid is not eliminated in significant amounts in urine or feces and the major metabolites exhibit lower antibacterial activity, significant antibacterial effects in the bladder or large intestine after oral or intravenous administration of tedizolid phosphate are not expected.

In summary, tedizolid, which is the active moiety and the most significant circulating metabolite of tedizolid phosphate, is eliminated primarily in feces as tedizolid sulfate conjugate; this stands in marked contrast to the oxidative metabolism and primarily renal excretion of linezolid and its metabolites. It is also noteworthy that the primary antibacterial agent, tedizolid, is derived from rapid metabolic conversion of a highly soluble phosphate prodrug, which facilitates oral

### TABLE 6

Mean metabolite percentage of administered dose in excreta

<table>
<thead>
<tr>
<th></th>
<th>Urine</th>
<th>Feces</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>Desmethyl tedizolid</td>
<td>1.069</td>
<td>0.697</td>
<td>N.D.</td>
</tr>
<tr>
<td>Carboxy tedizolid</td>
<td>3.583</td>
<td>0.831</td>
<td>4.188</td>
</tr>
<tr>
<td>Tedizolid sulfate</td>
<td>10.197</td>
<td>2.386</td>
<td>69.117</td>
</tr>
<tr>
<td>Tedizolid</td>
<td>1.017</td>
<td>0.415</td>
<td>1.963</td>
</tr>
</tbody>
</table>

N.D., not detected; S.D., standard deviation (for 6 subjects).

![Fig. 4. Proposed biotransformation of tedizolid phosphate. Putative metabolites were identified by mass spectrometry.](image)
absorption. Tedizolid exhibits high protein binding, a large volume of distribution, and an overall PK profile that supports the use of a once daily dosing regimen (i.e., 200 mg tedizolid phosphate, either oral or intravenous) currently under investigation in clinical trials for treating serious bacterial infections.

### Authorship Contributions

**Participated in research design:** Bartizal, Prokocimer.

**Conducted experiments:** Locke.

**Performed data analysis:** Ong, Flanagan, Fang, Dreskin, Locke, Bartizal, Prokocimer.

**Wrote or contributed to the writing of the manuscript:** Ong, Flanagan, Fang, Dreskin, Locke, Bartizal, Prokocimer.

### References


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