Metabolism, Excretion, and Pharmacokinetics of [14C]Tigecycline, a First-In-Class Glycylcycline Antibiotic, after Intravenous Infusion to Healthy Male Subjects

Matthew Hoffmann, William DeMaio, Ronald A. Jordan, Rasmy Talaat, Dawn Harper, John Speth, and JoAnn Scatina

Departments of Biotransformation (M.H., W.D., R.A.J., R.T., J.A.S.), Clinical Research (J.S.), and Clinical Pharmacology (D.H.)
Wyeth Research, Collegeville, Pennsylvania

Received March 8, 2007; accepted May 25, 2007

ABSTRACT:

Tigecycline, a novel, first-in-class glycylcycline antibiotic, has been approved for the treatment of complicated intra-abdominal infections and complicated skin and skin structure infections. The pharmacokinetics, metabolism, and excretion of [14C]tigecycline were examined in healthy male volunteers. Tigecycline has been shown to bind to bone; thus, to minimize the amount of radioactivity binding to bone and to maximize the recovery of radioactivity, tigecycline was administered intravenously (30-min infusion) as a single 100-mg dose, followed by six 50-mg doses, every 12 h, with the last dose being [14C]tigecycline (50 μCi). After the final dose, the pharmacokinetics of tigecycline in serum showed a long half-life (55.8 h) and a large volume of distribution (31.0 l/kg), whereas radioactivity in serum had a shorter half-life (6.9 h) and a smaller volume of distribution (3.3 l/kg). The major route of elimination was renal excretion of unchanged drug and metabolism to glucuronide conjugates and N-acetyl-9-aminomycinocycline. The glucuronide metabolites accounted for 5 to 20% of serum radioactivity, and approximately 9% of the dose was excreted as glucuronide conjugates within 48 h. Concentrations of N-acetyl-9-aminomycinocycline were approximately 6.5% and 11% of the tigecycline concentrations in serum and urine, respectively. Excretion of unchanged tigecycline into feces was the primary route of elimination, and the secondary elimination pathways were renal excretion of unchanged drug and metabolism to glucuronide conjugates and N-acetyl-9-aminomycinocycline.

Tigecycline [GAR-936, (4S,4aS,5aR,12aS)-9-[(2-tert-butylaminoacetyl)amino]-4,7-bis-dimethylamino-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,6,11,12a-octahydroporphine-2-carboxamide] (Fig. 1), a novel, first-in-class glycycycline and an analog of the semisynthetic antibiotic minocycline, is a potent, broad-spectrum antibiotic that acts by inhibition of protein translation in bacteria. Glyycycline antibiotics, including tigecycline and others (N,N-dimethylglycylamido-9-aminomycinocycline and N,N-dimethylglyclylamo-9-amin-6-demethyl-6-deoxytetracycline), are active in vitro against multiple antibiotic-resistant pathogenic bacteria, including meticillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci (Fraize et al., 1995; Weiss et al., 1995). Tigecycline is also active against bacterial strains carrying the tetracycline ring structure, such as doxycycline, tetracycline, chlorotetracycline, and demethylchlotetracycline, generally undergo little or no metabolism (Kelly and Buyske, 1960; Kelly et al., 1961; Eisner and Wulf, 1963; Swarz, 1976; Nelis and De Leenheer, 1981). Minocycline is an exception to this pattern, since it undergoes metabolism in humans via hydroxylation and N-demethylation (Nelis and De Leenheer, 1982; Böcker et al., 1991).

The pharmacokinetic profile of tigecycline in healthy human subjects is characterized by a long half-life of 37 to 67 h and a large volume of distribution (VSS) of 7 to 10 l/kg (Muralidharan et al., 2005). The metabolism of glycycycline antibiotics has not previously been investigated in humans. However, other antibiotics containing the tetracycline ring structure, such as doxycycline, tetracycline, chlorotetracycline, and demethylchlotetracycline, generally undergo little or no metabolism (Kelly and Buyske, 1960; Kelly et al., 1961; Eisner and Wulf, 1963; Swarz, 1976; Nelis and De Leenheer, 1981). Minocycline is an exception to this pattern, since it undergoes metabolism in humans via hydroxylation and N-demethylation (Nelis and De Leenheer, 1982; Böcker et al., 1991).

The present study was conducted to evaluate the pharmacokinetcia, metabolic disposition, and mass balance of a 50-mg intravenous dose of 14C-labeled tigecycline in healthy male volunteers. 14C-Tigecycline has been shown to distribute extensively into bone following a single i.v. dose to rats, with bone to plasma ratios for radioactivity as high as 2000 (Tombs, 1999). Even with this extensive distribution into bone, the estimated exposure to radioactivity after a 50-μCi (50-mg) intravenous dose of 14C-tigecycline to humans was 0.023 rem or 0.46% of the maximum allowable exposure from radioactive drugs for

ABBREVIATIONS: LC-MS/MS, liquid chromatography-tandem mass spectrometry; HPLC, high performance liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; AUC, area under the curve; AUCt, area under the concentration-time curve; HMBC, heteronuclear multiple bond correlation; MRT, mean residence time; CL, clearance.
human research subjects (5 rem). However, to minimize the amount of 
14C-labeled material binding to bone and to maximize the recovery of 
radioactivity, the 14C-labeled tigecycline dose was administered after 
multiple doses (350 mg over 3 days) of unlabeled tigecycline. Using 
this dosing regimen, previous studies have shown that steady-state 
tigecycline serum concentrations were achieved on day 4 (Sun et al., 
2005). Thus, for the current study, it was assumed that by the time of 
the 14C-tigecycline dose, steady-state or close to steady-state conditions 
had been achieved in serum, as well as in other tissues such as bone.

Materials and Methods

Materials. Tigecycline, as a lyophilized powder in vials containing a 50-mg 
dose of drug, was provided by Wyeth Research (Collegeville, PA). The manufacturing and packaging of 14C-tigecycline were carried out at the 
Parenteral Medications Laboratories, College of Pharmacy, University of 
Tennessee Health Science Center (Memphis, TN). Nonradiolabeled tigecycline 
drug substance was used to dilute radiolabeled tigecycline to make the final 
14C-drug substance. Radiolabeled tigecycline for injection was prepared in 
5-mL clear glass vials, each containing 53 mg of lyophilized, sterilized free 
tigecycline powder. The final 14C-tigecycline drug product had a specific 
activity of 9.2 mCi/mmol (1.00 µCi/mg) with a radiochemical purity of 98.6% 
and a chemical purity of 99.2%. Appearance, strength, identity, specific activity, and purity testing of 14C-tigecycline for injection was performed by 
ABC Laboratories (Columbia, MO). Bacterial endotoxin and sterility testing of 
14C-tigecycline for injection was carried out by Wyeth Research.

An additional batch of 14C-tigecycline (95.3 µCi/mg, 97.2% radiochemical purity), used in control samples, and [7-butyld-9]tigecycline, the internal 
standard for the determination of tigecycline in serum and urine, were received from 
GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). Tigecycline reference standard (98.4% chemical purity), 9-aminominocycline-hydrochloride 
salt reference standard (CL-318614; 97% chemical purity), and N-acetyl- 
9-aminominocycline (WAY-188749, batch L23566-162) were received from 
Wyeth Research. All other reagents and chemicals were obtained from 
commercial sources.

Study Design. This open-label, inpatient, multiple-dose tigecycline, single-
dose 14C-tigecycline metabolic disposition and mass balance study was per-
fomed in healthy men. Eligible subjects were selected on the basis of inclu-
sion/exclusion criteria, medical history, physical examination, and additional 
procedures outlined in the study protocol. Subjects using any investigational or 
commercial sources.

Dose Administration. The study was initiated with 12 healthy adult male 
volunteers to ensure six subjects would receive the radiolabeled dose. Each 
subject received a 100-mg loading dose on the morning of day 1, followed by 
a 50-mg maintenance dose every 12 h for an additional five doses. On the 
subject received a 100-mg loading dose on the morning of day 1, followed by 
volunteers to ensure six subjects would receive the radiolabeled dose. Each 
gave written informed consent before the screening process was initiated.

Throughout the study, subjects received a high fiber meal approximately 2 h 
before tigecycline administration. Study subjects received two tablets of Fi-
berCon (Wyeth) daily from day 1 through day 13 to facilitate regular bowel 
movements. The dosing and “inpatient-confinement portion” of the study were 
conducted at Quintiles (Kansas City, MO). Aliquots of the 14C-tigecycline 
dosing solution obtained before and after dose administration, dosing vials, 
and dosing apparatus were shipped to ABC Laboratories for radioactivity analysis.

Clinical Sample Collection. Whole blood samples (3 ml) from each sub-
ject, for the determination of total radioactivity, were collected into Vacutainer 
tubes (BD, Franklin Lakes, NJ) containing EDTA within 2 h before 14C-tige-
cycline dosing and approximately 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 
144, 168, 192, 216, and 240 h after the 14C-tigecycline dose. Additional whole 
blood samples (7 ml) were collected within 2 h before the first tigecycline dose 
and at the same collection times as the 3-ml whole blood samples. From these 
whole blood samples, serum was obtained after clot formation and centrifu-
gation of the sample, and used to determine tigecycline and total radioactivity 
concentrations in serum. Whole blood samples (50 ml) for serum collection 
were drawn approximately 2 h before the first tigecycline dose, within 2 h 
before 14C-tigecycline dosing and approximately 1, 4, 8, 24, and 48 h after the 
14C-tigecycline dose. These serum samples were stored at −70°C until analyzed 
for metabolite profiles.

Urine samples were collected within 2 h before the 14C-tigecycline dose 
and all urine was collected from study subjects after the 14C-tigecycline dose at intervals of 0 to 4, 4 to 8, and 8 to 24 h and every 24 h thereafter up to 240 h. Samples were kept refrigerated during the collection intervals and then 
were separated into samples for total radioactivity determination, tigecycline 
centration determination, and metabolite profiling (samples up to 48 h after the 
14C-tigecycline dose), and stored at −20°C until analysis.

When possible, a fecal sample was collected from subjects before the 
14C-tigecycline dose, and all fecal samples up to 240 h after the 14C-tige-
cycline dose were also collected. Samples were homogenized in 3 volumes of 
water using a Stomacher (3500; Seward Limited, London, UK) and stored in 
plastic containers at 54°C until analysis. Aliquots of each sample were 
analyzed for total radioactivity concentrations and samples collected up to 48 h 
after the 14C-tigecycline dose were analyzed for metabolite profiles.

For two subjects, there were incomplete fecal collections following the 
14C-tigecycline dose, due to noncompliance, and another subject withdrew 
from the study after sample collections at 24 h. Data from these subjects were 
not used in the mass balance portion of the study, but were used in the 
pharmacokinetic analysis and metabolite profiling portions of the study.

Radioanalysis. All radioactivity determinations were made using either a 
Tri-Carb model 3100TR liquid scintillation counter (PerkinElmer, Wellesley, 
MA) or Beckman LS6000SC or LS6500 liquid scintillation counters (Beck-
mann Coulter, Inc., Fullerton, CA). For dose, serum, and urine analysis, Ultima 
Gold scintillation fluid (PerkinElmer) was added to a known volume or weight 
of sample. Samples were then directly analyzed by liquid scintillation count-
ing. Fecal homogenates were weighed, allowed to dry, and combusted using a 
model 307 sample oxidizer (PerkinElmer). The resultant 14CO2 was trapped in 
Carbosorb in combination with Permafluor and radioassayed by liquid 
scintillation counting. For all sample matrices, a quench curve was used to 
concentrate using values to dose doses.

Measurement of Tigecycline in Serum and Urine. Serum and urine 
tigecycline concentrations were determined using validated LC-MS/MS as-
says. For serum, the assay was validated at concentrations between 10 
and 2000 ng/ml. In brief, tigecycline and an internal standard (7-butyld-
9)tigecycline were extracted from serum using 0.1% trifluoroacetic acid in 
aetonitrile. The denatured protein was removed by centrifugation and the 
supernatant was removed and evaporated to dryness at 35°C under a stream of 
air. Samples were reconstituted in 200 µl and an aliquot was injected for LC-MS/MS analysis using a Thermo Fisher Scientific (Waltham, MA) AquaSIL 
C18, 50 × 2.1 mm, 5-µm analytical column. Tigecycline and the internal 
standard were measured using the transition of the positive ions m/z 586.5→513.2 and 593.5→514.3, respectively. For the quality control samples, 
the overall precision (CV) was ≤10.5% and accuracy ranged from 104 to 
108%.

For urine, the assay was validated at concentrations between 0.20 and 80 
µg/ml. In brief, tigecycline and an internal standard in urine were diluted; the 
samples were mixed and transferred to fresh tubes, and an aliquot was injected 
for LC-MS/MS analysis using a Thermo Fisher Scientific AQUASIL C18, 
100 × 2.0 mm, 5-µm analytical column. For the quality control samples, the 
overall precision (CV) was ≤11.3% and accuracy ranged from 90.4 to 100%.

Metabolite Profiling by HPLC. Individual serum samples collected 1, 4,
and 8 h after the [14C]tigecycline dose were analyzed for metabolite profiles. The 24- and 48-h samples were not analyzed because the concentration of radioactivity was too low. Each serum sample was divided into two samples of equal volume (approximately 9 ml each) to provide duplicate analyses. EDTA (final concentration of 40 mM) and 3 volumes of acetone were then added to the samples. Samples were mixed and then centrifuged to remove the denatured protein. The supernatant was transferred to a fresh tube containing 20 µl of glacial acetic acid. The pellet was reextracted with 2 ml of water, 160 µl of 0.2 M EDTA, and 6 ml of acetone and processed as described above. The supernatants were combined and the pellet extraction was repeated. Supernatant samples were evaporated to dryness under a stream of nitrogen at room temperature. The residue was reconstituted in 300 µl of acetonitrile/water (1:9). The reconstituted residue was centrifuged to remove particulates and the supernatant transferred to an autosampler vial. The supernatant was assayed for radioactivity content and analyzed for metabolite profiles by HPLC. Extraction of control serum or fecal samples spiked with varying concentrations of [14C]tigecycline showed that the extraction process resulted in a reproducible percentage of tigecycline in samples being converted to its epimer during the extraction process. Therefore, the amount of epimer in the samples before extraction could be determined based on the amount of epimer in the final serum or fecal extracts.

Aliquots (1 ml) of the individual urine samples collected up to 48 h after the [14C]tigecycline dose were transferred to clean tubes and EDTA (final concentration of 40 mM) was added. The pH of the urine remained constant at pH 4.5 to 5.5. Samples were mixed and centrifuged, and the supernatants were transferred to autosampler vials. Separate aliquots of the supernatant were assayed for radioactivity content and analyzed for metabolite profiles by HPLC.

Fecal homogenates prepared from samples collected within 48 h after the [14C]tigecycline dose and containing greater than 8000 dpm/g were analyzed for metabolite profiles. Aliquots of the fecal samples (approximately 1 g) were transferred to 15-ml tubes, 3 volumes of acetone were added, and the samples were mixed and centrifuged. The supernatant was transferred to a fresh tube, and the pellet was resuspended with 40 mM EDTA in water and reextracted as described above. The pellet was reextracted in this manner a total of three times and the supernatants were pooled. The combined supernatants were evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 500 µl of water and centrifuged to remove particulates, and the supernatant was assayed for radioactivity content and analyzed for metabolite profiles by HPLC.

For LC/MS quantitation of metabolites M5 (N-acetyl 9-aminominocycline) and its epimer M4 in serum and urine samples from three subjects, standard curves were generated for each metabolite in each matrix. In serum, the standard curves ranged from 2.1 to 514.0 ng/ml for M4 and 2.8 to 568.6 ng/ml for M5, whereas in urine, the ranges were 5.7 to 632.5 ng/ml for M4 and 4.3 to 367.8 ng/ml for M5. For quantitation of these metabolites, urine samples were prepared in the same manner as those for metabolite profiling. For the serum samples, EDTA (final concentration of 40 mM) was added to each sample and the samples were vortex-mixed. One volume of acetonitrile was then added to each sample, samples were vortex-mixed, and denatured protein was separated by centrifugation. The supernatant was transferred to a fresh tube and concentrated under a stream of nitrogen to approximately 1 ml. The remaining supernatant was then assayed for M4 and M5 concentrations.

HPLC analyses for metabolite profiling were performed using a Waters 2695 Alliance Separation Module (Waters Corp., Milford, MA) with the sample temperature set to 4°C and a Waters model 2487 dual wavelength UV absorbance detector set to monitor 350 nm. The system was in-line with a Gilson 215 liquid handler (Gilson Inc., Middleton, WI) equipped to collect fractions at 20-s intervals. Fractions were collected into 96-well deep-well LumaPlates and analyzed for radioactivity using TopCount NXT (Packard Instruments, Meriden, CT). The absorbance detector was set to monitor 350 nm. The system was in-line with a 500 MHz Bruker DRX spectrometer equipped with a Cap-NMR flow cell operated in the positive ionization mode. The electrospray needle potential was set at 2.75 kV and the orifice potential was set at 44 V. The ion source was held at 80°C and the desolvation temperature was 250°C. In LC/MS experiments, the collision-activated dissociation of selected precursor ions was conducted using argon as the collision gas. The collision energy was 30 eV.

Serum extracts were analyzed for tigecycline and selected metabolites by LC-MS/MS in the selected reaction monitoring mode to reduce interference from endogenous components. These experiments were conducted with a dwell time setting of 200 ns. The following tigecycline-related components were monitored: tigecycline and its epimer, m/z 586→513; hydroxy tigecycline and tigecycline N-oxide, m/z 602→585 and m/z 602→472; N-desmethyl tigecycline, m/z 572→499; tigecycline glucuronide, m/z 762→569; 9-aminomino- cycline, m/z 473→456; and N-acetyl-9-aminomino-cycline, m/z 515→498. Urine and fecal samples were analyzed for tigecycline metabolites by LC-MS/MS analysis for identification of precursors of product ions characteristic of tigecycline. In addition, potential metabolites of tigecycline were searched for in the LC/MS data for subsequent MS/MS analysis.

The site of glucuronidation was further investigated using chemical oxidation of tigecycline and its glucuronide metabolite with Frensy’s salt (potassium nitrosodisulfonate). Frensy’s salt selectively oxidizes phenols, aromatic diols, aminophenols, and diamines to the corresponding quinones (Zimmer et al., 1971). The glucuronide metabolite used for these incubations was isolated from human urine following collection of the HPLC column effluent that contained the glucuronide metabolite. Three sets of incubations with Frensy’s salt were prepared: tigecycline alone, tigecycline plus the isolated glucuronide metabolite, and the isolated glucuronide metabolite alone. Incubations with tigecycline contained 10 µg/ml tigecycline in 10 mM ammonium acetate with 20 µM EDTA, and incubations with the tigecycline glucuronide contained the isolated tigecycline glucuronide with EDTA added (final concentration of 20 µM). Incubations were prepared in duplicate, and to one replicate Frensy’s salt was added, whereas to the other replicate nothing was added. Samples were incubated for at least 5 min at an ambient temperature of approximately 20°C before being directly analyzed by LC/MS.

Tigecycline glucuronide conjugates were synthesized using modified Koenigs-Knorr reaction conditions (Ag2CO3/MeCN in the presence of EDTA and molecule sieves) with an acyl-protected 1α-bromo-sugar as the donor to generate protected tigecycline glucuronides followed by de-protection in the presence of 0.1 N lithium hydroxide (in methanol/water 1:1). Cochromatography of the synthetic tigecycline glucuronides with tigecycline glucuronide (M7) isolated from human urine was used to determine the desired reaction product. NMR spectroscopy of the synthetic glucuronide was performed with 1H and 13C NMR spectroscopy. The glucuronide product. NMR spectroscopy of the synthetic glucuronide was performed with 1H and 13C NMR spectroscopy. The glucuronide

**Pharmacokinetic Analysis.** The radioactivity concentration data in serum (expressed in ng-Eq of tigecycline/ml), and the tigecycline serum concentration data for each subject were analyzed by using empirical, model-independ-
dent pharmacokinetic methods (Jusko, 1992). Peak concentration ($C_{max}$) was directly determined from the observed data. The apparent terminal-phase disposition rate constant ($\lambda_d$) for tigecycline in serum was estimated by a log-linear regression of the last three to seven observed concentrations that were determined to be in log-linear elimination by visual inspection. Due to low concentrations and rapid elimination, the concentration of radioactivity in serum could be determined only in samples collected within 24 h of dosing, and therefore, the $\lambda_d$ for this parameter was estimated using only two or three observed concentrations. The apparent terminal-phase disposition half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/\lambda_d$.

Since only one dose of [14C]tigecycline was administered, the serum radioactivity concentration data were analyzed as single-dose data. The single dose area under the concentration-time curve (AUC$_{0-t}$) to the last observable concentration ($C_{t}$) at time T was calculated by using the log-trapezoidal rule for decreasing concentrations and the linear-trapezoidal rule for increasing concentrations. The total single-dose AUC was estimated by $AUC = AUC_{0-t} + C_{t}/\lambda_d$. The single dose mean residence time (MRT) was calculated as $AUC/\text{AUMC}$, where AUMC is total area under the first moment curve and $T_{inf}$ is the duration of infusion (30 min).

The unlabeled tigecycline serum concentration data represent multiple-dose administration, and the steady-state AUC over one dose interval (AUC$_{0-t_{inf}}$, where $\tau = 12$ h) was calculated by using the log-trapezoidal rule for decreasing concentrations and the linear-trapezoidal rule for increasing concentrations. In addition, the steady-state MRT was calculated as $AUC_{0-t_{inf}}/AUMC_{0-t_{inf}} + \tau AUMC_{0-t_{inf}} - T_{inf}/2$. Systemic clearance (CL) was calculated as dose/AUC, and the apparent steady-state volume of distribution ($V_{SS}$) was estimated as $\text{CL} \cdot \text{MRT}$.

**Calculations.** The amount of total radioactivity (dpm) in the urine, feces, and serum was determined by multiplying the weight of samples by the radioactivity concentration (dpm/g) of each sample. The dose recovered was determined by total dpm in the sample at any given time point, divided by total radioactivity (in dpm) of the dose received by each subject, and multiplied by 100%. These calculations and the calculations of means and standard deviations were performed using Microsoft Excel 2000 software (Microsoft, Redmond, WA). If the dpm in any postdose sample aliquot was less than or equal to 2 times dpm in the predose sample aliquot (background), the value was presented as not detectable (N.D.).

The concentration of tigecycline-related components in serum, urine, and feces was calculated based on the total radioactivity concentrations. These concentrations were converted to tigecycline equivalent using the specific activity of the dose (1.00 μCi/mg). Using this value, the concentrations of the specific components were then estimated based on the distribution of radioactivity in the radiochromatograms. These metabolite concentrations reflect only the disposition of the final [14C]tigecycline dose and do not account for tigecycline or its metabolites remaining from the unlabeled doses. For the concentration determinations of the nonradiolabeled metabolites M4 and M5, the LC/MS peak area from study samples was compared with a standard curve generated using peak areas of control samples spiked with synthetic standards of M4 and M5.

**Results**

**Excretion of Radioactivity.** The final [14C]tigecycline intravenous dose contained 45.9 ± 0.9 μCi (mean ± S.D.), equivalent to 45.9 ± 0.9 mg. Recovery of radioactivity in urine and feces was calculated for five subjects (Table 1), with the excluded subject having withdrawn from the study after the 24-h sample collections. For two subjects who had incomplete sample collection, the recovery of radioactivity was <55% and these subjects were not used in the final mass balance calculations. The overall recovery of radioactivity by 240 h after administration of [14C]tigecycline was 91.8 ± 5.6% (mean ± S.D., n = 3), with feces being the major route of elimination. The majority of the radioactive dose (87.5 ± 5.1%) was eliminated within 120 h after dosing (Fig. 2).

**Pharmacokinetic Analysis.** The concentration-time curve and pharmacokinetic parameters for tigecycline in serum following the final tigecycline dose were different from those for radioactivity in serum (Table 2, Fig. 3). The tigecycline concentrations in serum represent tigecycline present from all doses, whereas the radioactivity measurements represent only the final, radiolabeled dose of tigecycline. Tigecycline in serum had a long $\lambda_d$ and a large $V_{SS}$, and tigecycline concentrations were measurable for 7 to 9 days after the final dose. Tigecycline CL from serum was 22 l/h (0.29 l/h/kg), whereas mean renal clearance (CL$_r$) was 4.6 l/h (data not shown). For

**TABLE 1**

Elimination of radioactivity in urine and feces following a single intravenous 50-mg dose of [14C]tigecycline

<table>
<thead>
<tr>
<th>Subject No.*</th>
<th>Mean (n = 5)</th>
<th>Mean (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.3 ± 1.9</td>
<td>33.2 ± 1.9</td>
</tr>
<tr>
<td>2</td>
<td>32.3 ± 1.9</td>
<td>33.2 ± 1.9</td>
</tr>
<tr>
<td>3</td>
<td>32.3 ± 1.9</td>
<td>33.2 ± 1.9</td>
</tr>
<tr>
<td>4</td>
<td>32.3 ± 1.9</td>
<td>33.2 ± 1.9</td>
</tr>
<tr>
<td>5</td>
<td>32.3 ± 1.9</td>
<td>33.2 ± 1.9</td>
</tr>
</tbody>
</table>

*Subject 4 withdrew from the study after the 24-h sample collection and recovery was not determined for this subject.

a Values exclude data from subjects 1 and 2 because of incomplete sample collection due to noncompliance.

b Subject 4 withdrew from the study after the 24-h sample collection and recovery was not determined for this subject.
Radioactivity in serum, the t_{1/2} was much shorter and the V_{SS} smaller than for tigecycline in serum. In addition, both CL of radioactivity from serum (41 l/h, 0.54 l/h/kg) and CL_{R}, (13 l/h, data not shown) were significantly higher than for tigecycline. Radioactivity concentrations in serum were measurable for approximately 12 to 24 h after the radiolabeled dose. The blood/serum ratios of radioactivity were between 0.7 and 1.1 at all time points tested (data not shown), which indicated no preferential binding of radioactivity to whole blood constituents.

Metabolic Profiles. HPLC chromatograms of extracted serum, urine, and fecal homogenate from a representative subject are shown in Fig. 4. The metabolite profiles were similar between subjects and at the different time points. Unchanged [^{14}C]tigecycline was the predominant radiolabeled component in the serum samples at all time points (Table 3). The values for tigecycline and the epimer of tigecycline take into account the amount of tigecycline that was converted to the epimer during the extraction process, as described under Materials and Methods. In serum, M1 (t-butylicyclic acid) and the glucuronide conjugates of tigecycline and its epimer (M3 and M2, respectively) increased in abundance, relative to tigecycline, from 1 to 8 h after the radioactive dose. Since the radiolabel had been cleaved from N-acetyl-9-aminominocycline (M5) and its epimer (M4), the metabolites were detected and quantified in serum and urine by LC/MS analysis (Table 4). Therefore, the amount of M4 and M5 detected was the result of all of the tigecycline doses and not just the final labeled dose. Metabolite M5 was detected in all serum samples analyzed at concentrations approximately 5 to 7% of the tigecycline concentrations in the same serum samples. The trace metabolite 9-aminominocycline (M6) was detected by LC/MS analysis of the serum, but was not quantified.

In urine collected up to 48 h after the [^{14}C]tigecycline dose, the predominant radiolabeled component was unchanged [^{14}C]tigecycline (Table 5). The epimer of tigecycline represented 2% of the radioactive dose and increased relative to tigecycline, over time. Some of the epimer may have formed in the bladder, since stability analysis demonstrated that tigecycline degraded to the epimer when incubated in human urine at 37°C (data not shown). In the 0- to 48-h urine samples, M1 represented 6.3% of the radioactive dose, whereas M3 represented 4.1%; M2 was not detected in urine. Within 48 h of the final tigecycline dose, approximately 1.5 mg of unlabeled metabolites M4 and M5 had been excreted in urine (Table 4). Additional trace metabolites in urine, detected by LC/MS, were 9-aminominocycline (M6) and two hydroxy tigecycline metabolites (M7 and M8).

For the fecal samples collected up to 48 h after the [^{14}C]tigecycline dose, tigecycline was the predominant drug-related component (Table 5). The epimer of tigecycline, glucuronide conjugates of tigecycline and its epimer (M4 and M5), and t-butylicyclic acid (M1) were all observed in feces. Additional uncharacterized radioactive peaks were observed in some fecal samples and represented less than 2.5% of the radioactive dose. The unlabeled metabolites 9-aminominocycline (M6), N-acetyl-9-aminominocycline (M5), and its epimer (M4) were not detected in any fecal samples.

Metabolite Structural Characterization. Figure 5 shows the proposed structures for each of the metabolite peaks observed by HPLC with radioactivity flow detection and/or LC/MS. Tigecycline generated a protonated molecular ion, [M + H]^+, at m/z 586. The product ions of m/z 586 mass spectrum included m/z 569, 513, 456, 211, 154, 126, and 86 (Fig. 6; Table 6). Loss of NH3 from [M + H]^+ generated m/z 569. The product ion at m/z 513 represented loss of the t-butylicyclic group from the t-butylicyclic aminoacidamino side chain. Loss of the entire t-butylicyclicaminoacid group from the t-butylicyclic aminoacidamino side chain generated m/z 456. The product ions at m/z 211, 154, and 126 originated from the A ring of the tetracycline ring system as indicated in the fragmentation scheme. The product ion at m/z 86 represented the t-butylicyclic aminoacidamino group. This proposed fragmentation scheme was consistent with...
the reported fragmentation of structurally similar antibiotics (Kamel et al., 2002).

The epimer of tigecycline generated a $[M+H]^+$ at $m/z$ 586. Its product ion of $m/z$ 586 mass spectrum was identical to the mass spectrum for tigecycline (Table 6). Identification as the epimer was made based on its relative retention time being shorter than that of tigecycline.

The $[M+H]^+$ for M2 and M3 was observed at $m/z$ 762, which was 176 Da larger than tigecycline (Table 6). Neutral loss of 176 Da generated $m/z$ 586, also the $[M+H]^+$ for tigecycline, which indicated a glucuronide of tigecycline. Product ions at $m/z$ 569, 513, 456, 211, 154, and 86 were also observed for tigecycline and did not indicate the site of conjugation. The product ion at $m/z$ 551 was formed by fragmentation of ring B, which indicated that the hydroxy group at either position 10 or 12 was the site of glucuronidation. LC/MS analysis of the reaction product formed after chemical oxidation of tigecycline with Fremy’s salt showed that tigecycline was oxidized to the quinone product for tigecycline, with subsequent oxidation of the hydroxyl group at position 12 (Fig. 7). Consistent with the mechanism of oxidation by the nitrosodisulfonate radical generated from Fremy’s salt, the initial site of reaction was position 7 (Zimmer et al., 1971). Oxidation of the D ring phenol to a quinone required the presence of a free hydroxyl group at position 10 to generate the cyclohexadienone intermediates.

TABLE 3

<table>
<thead>
<tr>
<th>Time</th>
<th>Butylaminocetonic Acid (M1)</th>
<th>Glucuronide Metabolites (M2 and M3)</th>
<th>Tigecycline Epimer</th>
<th>Tigecycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>9.8 ± 3.2</td>
<td>0.7 ± 1.2</td>
<td>8.3 ± 2.1</td>
<td>81 ± 23</td>
</tr>
<tr>
<td></td>
<td>(14 ± 4.1)</td>
<td>(0.8 ± 1.3)</td>
<td>(12 ± 3.0)</td>
<td>(118 ± 33)</td>
</tr>
<tr>
<td>4 h</td>
<td>17 ± 4.5</td>
<td>12 ± 8.0</td>
<td>6.8 ± 3.7</td>
<td>64 ± 29</td>
</tr>
<tr>
<td></td>
<td>(13 ± 5.5)</td>
<td>(8.4 ± 5.5)</td>
<td>(5.0 ± 2.7)</td>
<td>(47 ± 21)</td>
</tr>
<tr>
<td>8 h</td>
<td>19 ± 6.2</td>
<td>15 ± 10</td>
<td>5.4 ± 2.1</td>
<td>58 ± 28</td>
</tr>
<tr>
<td></td>
<td>(8.2 ± 2.8)</td>
<td>(7.4 ± 5.8)</td>
<td>(2.3 ± 0.9)</td>
<td>(25 ± 12)</td>
</tr>
</tbody>
</table>

*Corrected for the epimer formed during the extraction process.
The hydroxyl group at position 12 was subsequently oxidized to a keto group, but this would not have occurred without prior oxidation of the D ring phenol to a quinone. After incubations of M3 with Fremy’s salt, no reaction products were observed. If the 10-hydroxyl group were the site of glucuronidation, then oxidation products of tigecycline glucuronide (M3) would be expected. However, if the 12-hydroxyl group were the site of glucuronidation, then oxidation products of tigecycline glucuronide (M3) would be expected. The inability of tigecycline glucuronide to be oxidized by Fremy’s salt indicated that a free phenolic hydroxyl group was no longer present at position 10 on account of position 10 being the site of glucuronidation. In the 1H NMR spectrum of the synthetic tigecycline glucuronide, the proton signal for the C10-OH expected at δ 12.3 ppm for tigecycline was absent (data not shown), suggesting a 10-O-glucuronide structure. This suggestion was supported by a three-bond long-range correlation between H11 and H8 (δ 8.26) to C10. Therefore, the synthetic tigecycline glucuronide and tigecycline glucuronide isolated from human urine (M3) were both identified as tigecycline 10-O-β-d-glucuronide. Metabolite M2 was proposed to be the epimer of M3 based on its HPLC retention time being earlier than that of M3.

Metabolites M4 and M5 were observed in serum and urine, and only by LC/MS. The [M + H]+ for these metabolites was observed at m/z 515, which was 71 Da smaller than tigecycline. The product ions of m/z 515 mass spectrum for M5 included m/z 498, 456, 154, 126, and 58 (Table 6). Mass spectral data for M4 and M5 were similar and indicated that the tetracycline ring was intact. The lack of radiochromatographic peaks for M4 and M5 was consistent with loss of the radiolabeled carbonyl group of the t-butylaminoacetyl amino acid side chain. The most likely mechanism for formation of M5 was amide hydrolysis to generate t-butylaminooxycetonic acid (M1, which contained the radiolabel) and 9-aminominocycline (no 14C label). N-Acytlylation of 9-aminominocycline was proposed to generate M5. The 58-Da difference between the m/z 456 product ion and molecular weight (514) was consistent with the presence of a nonradiolabeled acetylamino group and the observation of a trace amount and only by LC/MS due to loss of the radiolabel. M4 was not detected in urine.

For quantification of N-acetyl-9-aminominocycline (M5) and its epimer (M4), only samples from three subject were used.

---

### TABLE 4

<table>
<thead>
<tr>
<th>Matrix and Collection Time</th>
<th>M4</th>
<th>M5</th>
<th>Tigecycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum −2 h</td>
<td>N.D.</td>
<td>10.5 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155 ± 39.0</td>
</tr>
<tr>
<td>24 h</td>
<td>N.D.</td>
<td>7.4 ± 2.6</td>
<td>120 ± 18.2</td>
</tr>
<tr>
<td>48 h</td>
<td>N.D.</td>
<td>3.9 ± 1.3</td>
<td>77.1 ± 21.4</td>
</tr>
<tr>
<td>Urine 0−4 h</td>
<td>12.7 ± 10.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>124 ± 112</td>
<td>4420 ± 715</td>
</tr>
<tr>
<td>4−8 h</td>
<td>19.6 ± 9.58</td>
<td>181 ± 93.7</td>
<td>2720 ± 1215</td>
</tr>
<tr>
<td>8−24 h</td>
<td>67.7 ± 29.9</td>
<td>555 ± 255</td>
<td>4910 ± 1540</td>
</tr>
<tr>
<td>24−48 h</td>
<td>59.3 ± 33.7</td>
<td>476 ± 297</td>
<td>2920 ± 1150</td>
</tr>
<tr>
<td>Total Urine (0−48 h)</td>
<td>159 ± 77.6</td>
<td>1340 ± 695</td>
<td>15000 ± 2980</td>
</tr>
</tbody>
</table>

<sup>a</sup>Time is expressed relative to the final tigecycline dose.
<sup>b</sup>N.D. indicates that the metabolite was below the limit of detection (2.1 ng/ml).
<sup>c</sup>Values for serum are mean ± S.D. of concentration in serum (ng/ml).
<sup>d</sup>Values for urine are mean ± S.D. of amount of compound excreted in each urine collection (µg).

---

### TABLE 5

Percentage of total [14C]tigecycline dose excreted as tigecycline and tigecycline metabolites within 48 h of dosing

<table>
<thead>
<tr>
<th>Matrix and Collection Time</th>
<th>Percentage of Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1 Glucuronide Metabolites (M2 and M3)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine 0−4 h</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>4−8 h</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>8−24 h</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>24−48 h&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Total Urine (0−48 h)</td>
<td>6.3 ± 0.9</td>
</tr>
<tr>
<td>Feces (0−48 h)</td>
<td>1.5 ± 1.0</td>
</tr>
<tr>
<td>Total (0−48 h)</td>
<td>7.8 ± 0.7</td>
</tr>
</tbody>
</table>

<sup>e</sup>M3, but not M2, was detected in urine.
<sup>f</sup>For the 24- to 48-h urine, the total urine, the fecal samples, and the total n = 5, because of incomplete urine and fecal collections from one subject.
<sup>g</sup>Corrected for the epimer formed during the extraction process.

---

*METABOLIC DISPOSITION OF [14C]TIGECYCLINE IN HUMANS*

### 9-Aminominocycline (M6) was observed in serum and urine in trace amounts and only by LC/MS due to loss of the radiolabel. The [M + H]+ for 9-aminominocycline was observed at m/z 473. The product ions of m/z 473 mass spectrum included m/z 456 (Table 6), generated from loss of NH3, which was characteristic of
FIG. 6. Product ions of m/z 586 mass spectrum for tigecycline.

Fig. 5. Tigecycline-related compounds detected in human serum (S), urine (U), and feces (F). * indicates the site of the 14C label.

Fig. 6. Product ions of m/z 586 mass spectrum for tigecycline.
tigecycline-related compounds. In serum samples, this metabolite was observed by monitoring the $m/z$ 473→456 SRM transition (data not shown). Confirmation of the identification of 9-aminominocycline was obtained by comparison with a synthetic standard. 9-Aminominocycline was proposed to have been generated by amide hydrolysis of the $t$-butylaminoacetylamino side chain with radiolabeled $t$-butylaminoacetic acid as a byproduct. Metabolite M1 was observed in serum, urine, and feces, as an early eluting radiochromatographic peak. Isolation of this early eluting M1 peak from human urine and subsequent LC/MS analysis with normal phase HPLC resulted in observation of $[M+H]^+$ for M1 at $m/z$ 132 and 173, respectively (data not shown). This indicated a molecular weight of 131, consistent with the identification as $t$-butylaminoacetic acid.

Hydroxylated tigecycline metabolites M7 and M8 were observed in urine in trace amounts. These metabolites produced a $[M+H]^+$ at $m/z$ 602. Mass spectral data for both of the hydroxy metabolites were similar (Table 6). For both M7 and M8, product ions at $m/z$ 529 and 472 were 16 Da larger than the corresponding ions at $m/z$ 513 and 456, respectively, for tigecycline. These data indicated that the tetracycline ring was the site of metabolism rather than the $t$-butylaminoacetylamino group. For M8, the product ions at $m/z$ 211 and 154 indicated an unchanged A ring. Therefore, these metabolites were the product of oxidation of the tetracycline moiety.

**Discussion**

Intravenous administration of tigecycline for 3 days (total dose of 350 mg), followed by a single i.v. dose of $[^{14}C]$tigecycline (50 mg, 50 µCi), was well tolerated. The rationale for preceding the $[^{14}C]$tigecycline dose with multiple doses of nonradiolabeled drug was based upon the ability of tigecycline to bind to bone (Tombs, 1999). This study design was meant to minimize the amount of radioactivity binding to bone while maximizing the recovery of radioactivity. Another consequence of this study design was that the pharmacokinetic parameters for unlabeled tigecycline in serum were somewhat different from those for radioactivity in serum (Table 2). Tigecycline pharmacokinetics in serum for the current study, including $C_{\text{max}}$, $AUC$, $CL$, $V_{SS}$, and $t_{1/2}$, were similar to previously reported data under steady-state conditions (Muralidharan et al., 2005). Also consistent with previous data, the concentration versus time curve for tigecycline in serum, as well as for radioactivity in serum, appeared to be biphasic, with rapid distribution into tissues followed by a longer elimination phase, probably caused by the continual release of tigecycline from slowly equilibrating tissues such as bone. However, the
After intravenous administration of \(^{14}C\)tigecycline, unchanged drug was the predominant radiolabeled component in serum, urine, and feces, with more than half of the radioactivity excreted within 48 h of dosing identified as \(^{14}C\)tigecycline. The epimer of tigecycline was also observed in each matrix, accounting for approximately 5 to 10% of the radioactivity in serum, urine, and feces. The epimer of tigecycline was shown to be a degradant rather than a metabolite, inasmuch as it was formed when tigecycline was incubated in control human serum and urine at 37°C (data not shown). Epimerization at the C-4 position has also been reported for structurally similar antibiotics (Remmers et al., 1963; Nelis and De Leenheer, 1982).

Metabolites M3 (tigecycline glucuronide) and its epimer, M2, were observed in serum, urine (M3 only), and feces. These metabolites represented up to 15% of the radioactivity in serum, and nearly 10% of the dose was excreted as glucuronide conjugates within 48 h of dosing. There are no reports in the literature that identify glucuronidation as a metabolic pathway for antibiotics with similar structures. In fact, these structurally similar antibiotics generally undergo little or no metabolism in humans (Kelly et al., 1961; Eisner and Wulf, 1963; Swarz, 1976; Nelis and De Leenheer, 1981). One exception to this is minocycline, which is metabolized to 9-hydroxyminocycline, \(N\_7\)-demethylminocycline, and \(N\_7\)-demethylaminominocycline in humans (Nelis and De Leenheer, 1982). The 9-hydroxyminocycline and \(N\_7\)-demethylaminominocycline (site of demethylation not determined) metabolites can each represent up to 8% of the dose in urine (Böcker et al., 1991). Additional metabolites of minocycline have been reported in human urine, but these metabolites have not been characterized (Nelis and De Leenheer, 1982). No \(N\_7\)-demethyl metabolites of tigecycline were observed in the current study. Trace amounts of two hydroxy tigecycline metabolites were detected by LC/MS analysis in urine, but the site of hydroxylation and detection by radiochromatography were not possible because the metabolites were present at very low concentrations.

Metabolite M1 (\(t\)-butylaminoacetylamino acid) was observed in each biological matrix, accounting for up to 20% of the radioactivity in serum and approximately 8% of the dose as M1 within 48 h. Metabolism of tigecycline to M1, via amide hydrolysis of the \(t\)-butylaminocetylaminoglucono group, also led to the formation of 9-aminominocycline (M6, not radiolabeled). However, only trace amounts of M6 were detected in serum and urine. The difference in the amount of M1 and M6 observed can be accounted for because a large proportion of the M6 was further metabolized via N-acetylation to N-acetyl-9-aminominocycline (M5) and its epimer (M4). This metabolic pathway was demonstrated using human liver cytosol incubated with M6 (data not shown). Although both M5 and M6 demonstrated some antibacterial activity in vitro, their activities were generally less than that of tigecycline and were effective against a narrower range of infectious organisms (data on file at Wyeth Research). Metabolite M4 was not tested for antibacterial activity since epimerization at the C-4 position in tigecycline and structurally similar antibiotics significantly decreases activity (Zhanel et al., 2004).

It is important to recognize that the excretion data and most of the metabolite profiling performed in this study assess only the disposition of the final \(^{14}C\)-labeled tigecycline dose, and does not account for tigecycline-related products remaining from the unlabeled doses. Therefore, the estimated concentrations of the tigecycline-related products that were based on radioactivity concentration data may underestimate the actual concentrations of these components in serum, urine, and feces. The exception to this is the concentrations of the unlabeled metabolites M4 and M5, which were determined by LC/MS analysis rather than radioactivity concentrations. The amounts of \(^{14}C\)tigecycline in serum and urine reported are somewhat lower (generally 10 to 75% less) than the total tigecycline amounts reported. In addition, the contribution of the \(^{14}C\)tigecycline to the total
tigecycline amount in serum and urine decreased with time; as mentioned above, this is consistent with the last-in, first-out phenomenon.

In summary, following i.v. administration of tigecycline for 4 days (total dose of 400 mg), including a single i.v. dose of \([^{14}C]\)tigecycline (50 mg, 50 \(\mu\)Ci), excretion of unchanged tigecycline into feces was the primary route of elimination, and the secondary elimination pathways were renal excretion of unchanged drug, and metabolism to the glucuronide conjugates and the N-acetyl-9-aminominocycline metabolite. Although unchanged drug was the predominant radiolabeled component in serum, urine, and feces, three radiolabeled metabolites were detected by radiochromatography and a total of eight metabolites characterized by LC/MS.

Acknowledgments. We recognize and thank Julie Chang and Robin Moore for contributions to the metabolite profiling and characterization portions of this study and Oliver McConnell for contributions to the characterization of the glucuronide metabolite using chemical oxidation. We also thank Joan Korth-Bradley for insightful discussions and recommendations. We appreciate the contributions of Ping Cai and Jianxin Gu in identifying the structure of the tigecycline glucuronide using NMR spectroscopy.

References


Address correspondence to: Matthew Hoffmann, Wyeth Research, 500 Arcola Road/S3226, Collegeville, PA 19426. E-mail: hoffmam7@wyeth.com