Pharmacokinetics and excretion of dalbavancin in the rat

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Objectives: The pharmacokinetics, tissue distribution and excretion routes of dalbavancin, a semi-synthetic glycopeptide, were investigated in rats.

Methods: A 20 mg/kg intravenous dose of dalbavancin or [3H]dalbavancin was administered to rats in three studies. Concentrations of dalbavancin or drug-derived radioactivity were assessed in blood, plasma, tissues, bile, urine and faeces by HPLC-MS/MS, scintillation counting or microbiological methods.

Results: Dalbavancin decayed tri-exponentially in plasma with an apparent terminal t1/2 of 187 h (approximately 8 days). Dalbavancin has dual routes of elimination, with around two-thirds of the excreted drug-derived radioactivity being found in the urine and around one-third in the faeces. After 70 days, 44.2% and 22.3% of the drug-derived radioactivity had been recovered in the urine and faeces, respectively. Biliary excretion of drug-derived radioactivity accounted for over half of the radioactivity excreted faecally. At 70 days post-dose, <5% of the dose remained in the carcass, showing that drug elimination was complete.

Conclusions: Dalbavancin has a long t1/2 (approximately 8 days) in the rat and distributes widely throughout the body. It is not selectively retained in any single organ, tissue or blood component and is completely eliminated by both renal and non-renal routes in rats. These data were useful in designing and interpreting animal infection model studies used to select the dose for human studies.

Keywords: glycopeptides, tissue distribution, mass balance

Introduction

Dalbavancin is being developed for the treatment of serious infections caused by Gram-positive organisms including methicillin-susceptible and -resistant Staphylococcus aureus. Pharmacokinetic studies in animals are essential to the evaluation of data from in vivo infection models, necessary prerequisites for the clinical development of anti-infective compounds. They are critical to the choice of dosage regimen for clinical trials as they are usually predictive of drug concentrations and drug elimination pathways in humans. The following studies were designed to further characterize the pharmacokinetics of dalbavancin in the rat by determining plasma concentrations, drug distribution and routes of excretion.

Materials and methods

Antimicrobial agents

Dalbavancin was produced at Vicuron Pharmaceuticals (Gerenzano, Italy). Tritiated dalbavancin stock solution ([3H]dalbavancin in ethanol, radioactive concentration 1.5 mCi/mL, specific activity 11.4 mCi/mg) was supplied by Sibtech, Inc. (Newington, CT, USA).

Dalbavancin for intravenous (iv) administration was prepared by dissolving the drug in a sterile solution of 5% dextrose. [3H]Dalbavancin for iv administration was prepared by dissolving [3H]dalbavancin and non-tritiated dalbavancin in a sterile dextrose solution. The radioactive concentration of the dalbavancin dose formulation was determined by liquid scintillation counting (Packard Tri-Carb 1900CA, 1900TR or 2100TR, Packard Canberra Instruments, Mississauga, Canada).

Animals

All experiments were carried out in male Sprague–Dawley rats (Charles River Canada, St-Constant, Canada or Harlan Italy, S. Pietro al Natisone, Italy). For the mass balance study, animals were housed in metabolic cages (Techniplast, Buguggiate, Italy or Mini Mitter, Bend, OR, USA) for the separate collection of urine and faeces. All animals were kept under environmentally controlled conditions with 12 h cycles of light and dark. Animals were fed a standard certified laboratory diet (Teklad 7012C, Harla-Teklad, Indianapolis, IN, USA), and were provided with filtered sterile...
water ad libitum. Procedures were approved by the Animal Care Committee at Vicuron Pharmaceuticals (Gerenzano, Italy) or MDS Pharma Services (Montreal, Canada).

**Experimental design**

**Pharmacokinetic study.** A single 20 mg/kg dose of dalbavancin was administered as an iv bolus into the caudal vein of 13 rats. Urine was collected from four rats during the first 6 h following the dose, at 12 h intervals thereafter until 72 h, and then at 24 h intervals until 240 h post-dose. Blood samples (0.3–0.5 mL) were collected from the remaining nine animals under light halothane anaesthesia from the retro-orbital sinus into heparinized tubes and centrifuged at 5000g for 5 min to obtain plasma. Blood samples were collected from three animals per group at 18, 43, 66, 108, 156 and 204 h for one group; 0.33 (20 min), 3, 9, 55, 132, 180 and 228 h for a second group; and at 0.083 (5 min), 0.5, 1, 30 and 84 h for a third group. These times were chosen to provide a full pharmacokinetic profile and to match the urine collection time intervals. Plasma and urine were assayed for dalbavancin using HPLC with tandem mass spectrometry (HPLC-MS/MS).

**Quantitative tissue distribution study.** Forty rats received a single 30 min iv infusion of 20 mg/kg [3H]dalbavancin (approximately 50 μCi per animal) via an implanted jugular catheter. The bile duct was cannulated in four of these animals 5 days before dose administration. Following the dose, bile was collected at 0–4, 4–8 and 8–24 h and at 24 h intervals thereafter to 120 h; and at 360–384 h. These animals were killed after the last collection period. The other rats, three per group, were killed at the following post-dose times: 12, 24, 48, 72, 96, 120, 144, 168, 336, 840, 1176 and 1680 h.

Tissue, blood and plasma samples were collected from the animals post mortem and samples were assayed for drug-derived radioactivity as described below. Forty different types of tissue samples were collected in the study (Table 1).

**Mass balance study.** Fifteen animals received a single 30 min iv infusion of 20 mg/kg [3H]dalbavancin. Urine and faeces were collected at 24 h intervals up to 336 h (n = 5), 864 h (n = 5) or 1680 h (n = 5) post-dose, and when animals were killed. Urine, faeces, cage washings and selected carcasses were analysed for drug-derived radioactivity as described below.

**Bioanalytical methods**

**HPLC-MS/MS.** Plasma and urine obtained from the pharmacokinetic study were assayed for dalbavancin concentrations using HPLC-MS/MS.1 A Luna C18 column (Phenomenex, Torrance, CA, USA) and PE-SCIEX API III+ MS/MS system, equipped with a Turbo-Ion Sprayer Source and operating in positive ionization mode were used. BI-K0098, another semi-synthetic glycopeptide, was used as the internal standard (Vicuron Pharmaceuticals, Gerenzano, Italy). Plasma and urine samples were injected onto the HPLC-MS/MS system following protein precipitation with acetonitrile (Carlo Erba, Italy) and a 1:10 dilution with the internal standard. The lower limit of quantification (LLOQ) was 0.5 mg/L dalbavancin in rat plasma (linearity 0.5–50 mg/L) and 0.2 mg/L dalbavancin in rat urine (linearity 0.2–10 mg/L).

**Microbiological assay.** Dalbavancin concentrations in samples obtained from the plasma pharmacokinetic study were also assayed using an agar diffusion assay with *Bacillus subtilis* ATCC 6633 as the detector organism.2 Dalbavancin plasma concentrations were plotted against calibration curves prepared with blank plasma in the range 0.4–100 mg/L. The LLOQ for dalbavancin was 0.4 mg/L.

**Drug-derived radioactivity.** Drug-derived radioactivity was assayed in urine, faeces, cage washings and selected carcasses from the mass balance study, and from blood, plasma, bile and tissues collected in the quantitative tissue distribution study. Samples were homogenized, solubilized and/or decolourized as appropriate for each tissue matrix. Hionic Fluor (10 mL) was added to each sample and radioactivity determined by liquid scintillation counting. All samples were counted for 20 min or to 2σ (1%). Quench corrections were made by the external standard method and background radioactivity determined by analysis of tissue samples collected from the control animal. The LLOQ for radioactivity was considered to be twice background. Samples below the LLOQ were considered to be zero for subsequent calculations.

**Pharmacokinetic analyses**

Dalbavancin and drug-derived radioactivity concentrations were summarized by tissue/fluid and time from dose administration. WinNonlin Professional, version 3 (Pharsight, Mountain View, CA, USA), was used to calculate pharmacokinetic parameters. For urinary excretion, the cumulative urinary excretion and rate of excretion of dalbavancin were calculated. A model-independent estimate of the rate constant associated with the terminal phase of the rate of excretion time curve, λuz, was made by log-linear regression of the apparent mono-exponential terminal phase. Pharmacokinetic parameters estimated were: amount recovered in urine, percentage of dose recovered and renal clearance (ClR), which was estimated by dividing the amount recovered in urine by the plasma exposure.

For the rat pharmacokinetic study, the levels of dalbavancin in plasma following iv bolus administration were fitted to a three-compartment model according to the equation: 

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t},$$

where $C_p$ represents the dalbavancin concentration at time $t$, $A$, $B$ and $C$ are hybrid coefficients and $\alpha$, $\beta$ and $\gamma$ are the rate constants for absorption, distribution and elimination, respectively.
are hybrid first-order rate constants. The Gauss-Newton minimization algorithm with modifications by Hartley and Levenberg was used to fit the model to data using $1/y^2$ as a weighting factor. The goodness of fit was judged on residual plot inspection, F-test and the Akaike Information Criteria. The following pharmacokinetic parameters were also estimated using the model-dependent method: $C_{\text{max}}$, initial, second and terminal phase half-lives ($t_{1/2a}$, $t_{1/2b}$ and $t_{1/2g}$), AUC, CL, $V_s$, and the mean residence time (MRT).

A model-independent estimate of the pharmacokinetic parameters was carried out using the rate constant associated with the terminal phase of the plasma curve ($k_z$), calculated by log-linear regression of the apparent mono-exponential terminal phase. Pharmacokinetic parameters estimated by this method included $C_{\text{max}}$, $t_{1/2a}$, AUC, CL, $V_s$, and MRT.

The calculations of concentrations and percentage recoveries of radioactivity from animal tissues, faeces, urine and cage washings were carried out using DEBRA software, version 4.1b (Lablogic Systems Ltd, Sheffield, UK).

## Results

### Plasma pharmacokinetics

Dalbavancin plasma concentrations following a single 20 mg/kg iv bolus dose, measured by HPLC-MS/MS, microbiological assay and drug-derived radioactivity, demonstrated a similar pattern of plasma concentration decay. This suggests that no active metabolites are present in relevant amounts in plasma (Figure 1). Pharmacokinetic parameters determined by tri-exponential modelling and non-compartmental analyses (model-independent) are shown in Table 2. The disposition of dalbavancin was consistent with a three-compartment open model with elimination from the central compartment. The half-lives of the initial ($t_{1/2a}$), intermediate ($t_{1/2b}$) and terminal ($t_{1/2g}$) disposition phases were 0.18, 11.4 and 187.4 h, respectively. The AUC was 3194.2 mg·h/L, the $V_s$ was 0.52 L/kg, and the total clearance from plasma was 6.3 mL/h/kg.

### Tissue distribution

The quantitative tissue distribution study demonstrated wide distribution of drug-derived radioactivity across the 40 different types of tissues and fluids sampled. A plot of mean concentrations versus time for selected tissues, including those with the highest concentrations, is shown in Figure 2. By the first pharmacokinetic observation, 12 h after the infusion, all tissues had significant concentrations of radioactivity. The highest value was observed in most tissues (35/40) at the first time-point. Almost all tissues reached the maximum observed concentration within 24 h of the dose. The highest

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**Table 2. Plasma pharmacokinetic study parameters determined using the tri-exponential model and non-compartmental analyses**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tri-exponential model</th>
<th>Non-compartmental analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (mg/L)</td>
<td>339.02 (8.1%)</td>
<td>223.7 (4.6%)</td>
</tr>
<tr>
<td>CL (L/h/kg)</td>
<td>0.0063 (2.6%)</td>
<td>0.0065 (2.6%)</td>
</tr>
<tr>
<td>CLR (L/h/kg)</td>
<td>0.0024</td>
<td>-</td>
</tr>
<tr>
<td>$V_s$ (L/kg)</td>
<td>0.52 (13.9%)</td>
<td>0.38 (12.4%)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>82.94 (15.6%)</td>
<td>58.61 (14.6%)</td>
</tr>
<tr>
<td>AUC (mg·h/L)</td>
<td>3194.2 (2.6%)</td>
<td>3067.1 (2.6%)</td>
</tr>
</tbody>
</table>

CV, coefficient of variance.

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**Figure 1. Dalbavancin plasma concentrations following a single 20 mg/kg iv bolus dose to male Sprague–Dawley rats measured via HPLC-MS/MS, microbiological assay or via radiochemical methods. Data are expressed as the mean dalbavancin plasma concentration (or dalbavancin equivalent).**

**Figure 2. Dalbavancin mean concentrations in selected tissues versus time following a single 30 min iv infusion of 20 mg/kg [3H]dalbavancin.**

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**Figure 3.**
Concentrations of radioactivity were found in kidney and liver. Most tissues had higher levels than plasma by 3 days after the dose. For each tissue, recovered radioactivity after 5 days was less than 5% of the administered dose, suggesting that there was no selective retention of dalbavancin by any one tissue or organ. By day 14, five tissues (kidneys, liver, brown fat, skin and skeletal muscle) retained >1% of the radioactivity dose. By the terminal time-point, 70 days post-dose, total retained radioactivity in the carcass was <5%.

Concentrations of drug-derived radioactivity in skin were >5 μg eq/g throughout the first week following the dose. The concentrations of drug-derived radioactivity remained relatively low in the central nervous system, with corresponding tissue to plasma ratios <1 at all time-points. Blood to plasma ratios were approximately 0.6 and remained relatively constant over time.

**Excretion**

Ten days after a single unlabelled 20 mg/kg iv dose, the mean cumulative recovery of unchanged dalbavancin in urine was 34.5%. In another study, following a 20 mg/kg iv dose of [3H]dalbavancin, cumulative amounts of drug-derived radioactivity excreted into urine were 22.8%, 37.4% and 44.2% of the dose at 1, 36 and 70 days, respectively, post-dose. Corresponding recoveries in faeces were 3.7%, 17.4% and 22.3%, respectively. Total cumulative recovery of drug-derived radioactivity in bile by 16 days post-dose was 11%. Figure 3 summarizes the cumulative amounts of dalbavancin recovered in urine and drug-derived radioactivity recovered in urine, bile and faeces.

At 36 and 70 days post-dose, 6.4% and 4.5%, respectively, of the administered radioactive dose was recovered from the carcass. Negligible radioactivity was recovered in the cage washings (<1%). After 70 days of daily sampling, total recovery of radioactivity from urine, faeces, cage washings and carcass, not considering tritium exchange and exhaled water vapour, was >70%.

**Discussion**

This series of studies in rats has described the plasma concentration–time profile of dalbavancin and its distribution and fate following a single dose. Dalbavancin has a plasma concentration–time profile that decays multi-exponentially in plasma. Similar patterns of disposition, consistent with a three-compartment model with elimination from the central compartment, have been reported for other glycopeptides including teicoplanin. Results of pharmacokinetic investigations revealed that the total clearance of dalbavancin was 6.3 mL/h/kg and the apparent volume of distribution at steady-state was 0.52 L/kg, consistent with the observed wide distribution of dalbavancin throughout tissues. Dalbavancin has a terminal t1/2 of 124–188 h in the rat. However, most dalbavancin had cleared from the plasma by the start of the terminal phase of tri-exponential decay, indicating that the γ compartment is a slow equilibrating compartment. Nevertheless, this terminal phase could influence the concentrations of drug in peripheral tissues. Results showed that the β phase of tri-exponential decay of dalbavancin in plasma, characterized by a β t1/2 of 1.4 h, accounts for most of the plasma AUC. These findings have implications for determining time to steady-state of dalbavancin in plasma for design of multiple dose regimens in this species. Comparison of pharmacokinetic parameter estimates between compartmental and non-compartmental analysis showed only minor differences, due to the rather shallow profile of the terminal phase decay.

At 10 days post-dose, urinary excretion of intact dalbavancin had accounted for 34.5% of a 20 mg/kg dose. In addition, results of the mass balance study showed that approximately 25% of the total radioactivity was eliminated in the urine and faeces within 24 h, rising to approximately 50% within the first week: this is consistent with the plasma pharmacokinetic profile.

These studies show that dalbavancin is excreted via both renal and non-renal routes. The occurrence of either renal or hepatic impairment might affect the elimination of drugs that are eliminated solely via one route of excretion, leading to drug accumulation. If the dual routes of elimination are also observed in humans, dalbavancin may require fewer dosage adjustments in patients with such impairment.

The wide distribution and penetration of dalbavancin throughout tissues was confirmed in the [3H]dalbavancin tissue distribution study. Furthermore, the concentration and t1/2 of drug-derived radioactivity in skin were comparable to, or higher than, values observed in plasma, indicating that dalbavancin penetrates skin and other peripheral compartments. There was no retention of dalbavancin in any one tissue or organ. Furthermore, drug-derived radioactivity did not concentrate in blood or the cellular components of blood.

In conclusion, dalbavancin distributes widely throughout tissues, is eliminated in the rat via both renal and non-renal routes and has a t1/2 permitting infrequent dosing. These data were useful in selecting appropriate doses and timing of study assessments for animal infection model studies.

**Transparency declarations**

The authors were employees at Vicuron Pharmaceuticals, Inc. at the time this manuscript was written.
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References


