Distribution of Radioactivity in Bone and Related Structures following Administration of [^{14}C]Dalbavancin to New Zealand White Rabbits[▽]

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Received 8 January 2007/Returned for modification 2 March 2007/Accepted 27 May 2007

Penetration of dalbavancin into noninfected bone and joint tissues was assessed after an intravenous dose of 20 mg/kg (of body weight) [^{14}C]dalbavancin given to rabbits. Drug-derived radioactivity, determined over 14 days by either liquid scintillation counting or autoradiography, remained above the MIC for common gram-positive pathogens that cause bone and joint infections.

Infection of the bone and joint tissues can be a serious complication after orthopedic surgery and trauma. Gram-positive organisms, particularly *Staphylococcus aureus*, account for the majority of bone and joint infections, including osteomyelitis, septic arthritis, and prosthetic joint infection in hospitalized patients (2). Additionally, the high prevalence of antimicrobial-resistant pathogens, including methicillin-resistant *S. aureus* (MRSA), in hospitals worldwide is a therapeutic challenge (3). Effective treatment requires an antibiotic with proven efficacy and the ability to penetrate and persist at the site of infection.

Dalbavancin is a novel, semisynthetic lipoglycopeptide with potent in vitro activity against gram-positive bacteria including MRSA and intermediate-vancomycin-resistant *S. aureus* (1, 6, 7, 10). A regimen of two doses of dalbavancin administered 1 week apart was effective in the treatment of patients with complicated skin and soft-tissue infections with gram-positive bacteria (5). High levels of dalbavancin are sustained in human plasma due to its terminal elimination half-life of approximately 1 week (4), which allows for once-weekly administration of the drug. Hence, from a pharmacokinetic perspective, dalbavancin appears to have potential for use in infections that may require long-term therapy, such as bone and joint infections. The objective of this study was to quantitatively assess the penetration of dalbavancin into bone and joint tissues in rabbits.

(Research described in this paper was presented as a poster at the 16th European Congress of Clinical Microbiology and Infectious Diseases, Nice, France, 1 to 4 April 2006 [9].)

Twenty-one male New Zealand White rabbits (Hra: [NZW]SPF; Covance Research Products, Inc., Denver, PA), with a mean weight of 2.74 ± 0.17 kg and aged approximately 4 months, were randomly assigned to the control and treatment groups. Eighteen rabbits were injected via the marginal ear vein with a single dose of 20 mg [^{14}C]dalbavancin per kg body weight. The average dose administered was actually 19.86 mg/kg. This dose was selected in order to mimic plasma con-

centrations seen in humans. Three control animals received vehicle alone at a dose volume of approximately 2 ml/kg. At each scheduled time of necropsy (12, 24, 72, 120, 168, and 336 h after dosing), three animals from the treated group were euthanized. Control animals were euthanized at 336 h postdose. At each time point, samples of plasma, nucleus pulposus, bone marrow, and bone were collected from each animal, stored at -20°C , and protected from light until analysis. Plasma was collected within 1 h of blood sampling and stored at -20°C until analysis. As a secondary objective, cerebrospinal fluid (CSF) samples were collected as soon as possible following euthanasia. Bone and bone marrow samples were obtained from the right tibia of the rabbit. The marrow was extracted and stored, and the tibia was sectioned and stored separately. The intact left hind limbs (femur and tibia) from one rabbit per time point were also collected, skinned, and frozen in carboxymethyl cellulose blocks. The concentrations of drug-derived radioactivity were determined by liquid scintillation counting (LSC). All samples were counted in duplicate, and the lower limit of quantitation was three times the background count. The concentrations of drug-derived radioactivity in hind limb tissues were determined using quantitative autoradioluminography; the lower limit of quantitation was $0.14 \mu\text{g eq/g}$. Approximately four to six sections/limb ($\sim 40 \mu\text{m}$ thick) were collected using a cryomicrotome (Leica CM 3600; Leica Microsystems, Inc., Deerfield, IL) and maintained at -20°C . After drying, sections were mounted and exposed with ^{14}C -spiked blood calibration standards to ^{14}C -sensitive imaging plates (Molecular Dynamics, Sunnyvale, CA) at room temperature for 4 days. Sections were then removed, and the plates were scanned using the Typhoon 9410 image acquisition system (Molecular Dynamics, Sunnyvale, CA). Quantification, relative to the calibration standards, was performed by image densitometry using MCID image analysis software (Imaging Research, St. Catherine's, Ontario, Canada).

[^{14}C]dalbavancin-derived radioactivity was present in all samples analyzed over the 336-h period, except for CSF, as measured by LSC (Table 1). CSF concentrations were low and were measurable (just above the limit of quantitation) only at 12 h postdose ($0.08 \mu\text{g eq/g}$) and at 24 h postdose ($0.05 \mu\text{g eq/g}$). The radioactivity in plasma declined multiexponentially with a rapid initial phase followed by a slower elimination

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[▽] Published ahead of print on 4 June 2007.

TABLE 1. LSC data

Tissue	Concn of drug-derived radioactivity ($\mu\text{g eq/g tissue}$) at time point ^a :					
	12 h	24 h	72 h	120 h	168 h	336 h
Plasma	46.88 (6.65)	19.28 (1.21)	4.50 (0.40)	2.78 (0.22)	2.01 (0.35)	0.94 (0.09)
Bone	7.04 (0.15)	5.14 (0.81)	3.94 (0.64)	3.46 (0.39)	3.39 (0.26)	1.96 (0.14)
Bone marrow	15.14 (2.75)	14.75 (0.78)	12.74 (1.47)	9.52 (1.24)	16.08 (5.02)	12.02 (1.87)
NP ^c	0.86 (0.07)	0.76 (0.20)	0.67 (0.11)	0.75 (0.14)	0.64 (0.04)	0.60 (0.19)
CSF	0.08 ^b (0.00)	0.05 (0.01)	BQL ^d	BQL	BQL	BQL

^a Mean data (standard deviation); $n = 3/\text{time point}$.

^b Mean of two samples; the third sample was contaminated with a small amount of blood.

^c NP, nucleus pulposus.

^d BQL, below quantifiable limits of assay.

phase. Concentrations of radioactivity in bone marrow remained relatively constant over the course of the study and ranged between 15.14 $\mu\text{g eq/g}$ and 12.02 $\mu\text{g eq/g}$ at 12 h and 336 h postdose, respectively. Drug concentrations were more than three times higher in bone marrow than in plasma at 72 h postdose (12.74 $\mu\text{g eq/g}$ and 4.50 $\mu\text{g eq/g}$, respectively). Radioactivity levels in bone ranged from 7.04 $\mu\text{g eq/g}$ at 12 h postdose to 1.96 $\mu\text{g eq/g}$ at 336 h postdose. Post-hoc-derived area-under-the-curve values were 1,786 $\mu\text{g eq} \cdot \text{h/ml}$ for plasma and 1,125, 4,439, and 221 $\mu\text{g eq} \cdot \text{h/g}$ in bone, bone marrow, and nucleus pulposus, respectively. This resulted in tissue/plasma ratios of 0.63, 2.48, and 0.12 for bone, bone marrow, and nucleus pulposus, respectively.

Mean concentrations of [¹⁴C]dalbavancin-derived radioactivity were also measured by autoradioluminography (Table 2). Whole-blood levels of [¹⁴C]dalbavancin-derived radioactivity were highest at the first sampling point and then declined rapidly. At 12 h postdose, the dalbavancin-derived radioactivity was highest in bone marrow, followed by whole blood, articular cartilage, ligament, epiphyseal plate, periosteum, and meniscus. The lowest concentration was observed in compact bone. Tissue concentrations decreased steadily but slowly over the course of the study but remained relatively high in bone marrow, epiphyseal plate, periosteum, and articular cartilage. Figure 1 shows the pattern of radioactivity distribution in hind limb section tissues measured by autoradioluminography at 24, 120, and 336 h postdose. Concentrations were measurable in

all samples (except CSF) for at least 14 days following drug administration.

The differences in concentrations in bone and bone marrow measured by LSC and autoradioluminography may be due to the different sampling and analysis techniques. The LSC technique is subject to a number of inherent limitations that cause

TABLE 2. Autoradioluminography data

Tissue	Concn of drug-derived radioactivity ($\mu\text{g eq/g tissue}$) at time point ^a :					
	12 h	24 h	72 h	120 h	168 h	336 h
Blood (in vessel)	30.58	14.49	5.02	1.50	5.33	3.08
Periosteum	20.90	11.35	8.78	12.20	7.28	5.18
Articular cartilage	25.05	21.44	8.04	6.56	7.48	4.84
Compact bone	1.56	1.56	0.39	0.38	0.34	0.24
Epiphyseal plate	21.92	18.72	11.66	10.27	13.10	8.42
Ligament	22.12	8.64	4.56	3.68	2.67	1.43
Synovial space	6.56	4.25	1.49	2.66	2.66	1.16
Meniscus	19.19	12.44	8.04	4.39	NI ^b	NI
Bone marrow	40.20	32.07	37.79	29.95	18.90	30.27
Skeletal muscle	3.26	1.92	1.25	0.99	0.94	0.80

^a $n = 1/\text{time point}$. Lower limit of quantitation = 0.000685 $\mu\text{Ci/g}$ /0.0050029 = 0.1370 $\mu\text{g eq/g tissue}$.

^b NI, not identifiable on autoradioluminograph.

A) 24 h postdose

B) 120 h postdose

C) 336 h postdose

FIG. 1. Autoradioluminograph of the radioactivity distribution in the hind limb of a male albino rabbit after a single intravenous administration of [¹⁴C]dalbavancin at a target dose of 20 mg/kg.

variability in the amount of bone and bone marrow collected during sampling. These include the possibility of contamination from adjacent tissues and biological fluids during necropsy and during the homogenation and combustion processes. In contrast, tissue concentrations measured by autoradioluminography are subject to less variability because they are visualized from intact limbs. Therefore, autoradioluminography may be more likely to reflect actual tissue levels; however, the sample size of $n = 1$ limits this interpretation.

The metabolism of dalbavancin has not been studied in rabbits. However, it is not a substrate, inducer, or inhibitor of human hepatic cytochrome P450 isoenzymes. No significant amounts of metabolite have been observed in human plasma; however, a minor metabolite (hydroxydalbavancin), which accounts for 8 to 12% of the administered dose, has been observed in human urine. The metabolite has also been demonstrated in rat and dog urine (approximately 6% and 18% of the administered dose, respectively) (data on file at Pfizer Inc., New York, NY). Considering the limited metabolism of dalbavancin and the rapid elimination of the metabolite from the systemic circulation, it is likely that most of the drug-derived radioactivity observed in these experiments is associated with the parent drug.

In the present study, the concentration of drug-derived radioactivity in bone marrow at 336 h postdose was 12.02 $\mu\text{g eq/g}$ and 30.27 $\mu\text{g eq/g}$ as measured by LSC and autoradioluminography, respectively. The MICs for dalbavancin range from ≤ 0.015 to 0.5 mg/liter (MIC at which 90% of the isolates tested are inhibited, 0.06 mg/liter) for staphylococci and ≤ 0.015 to 0.25 mg/liter (MIC at which 90% of the isolates tested are inhibited, 0.03 mg/liter) for streptococci (2). Total drug concentrations above the MICs for key pathogens were maintained in the rabbit bone for up to 336 h. These experiments do not provide an assessment of the free dalbavancin in the bone that would be available for antibacterial activity. Hence, direct correlation of total (free and bound fraction) drug-derived radioactivity with MICs should be made cautiously.

Using autoradiography, Saleh Mghir et al. examined the penetration of teicoplanin following administration of [^{14}C]teicoplanin (20 mg/kg twice daily for 7 days) to rabbits with or without joint prostheses and after injection of either MRSA or saline in the joints (8). In the saline-injected animals without prostheses, the authors showed that the tissue/blood ratio of drug-derived radioactivity on day 15 in the bone, bone marrow, articular cartilage, epiphyseal disk, and ligament was less than 1, while in the periosteum it was slightly greater than 1. In the current study, drug-derived radioactivity on day 14 following administration of a single dose of dalbavancin was higher in the periosteum, articular cartilage, epiphyseal disk, and bone marrow than in blood, while it was lower in the

compact bone, ligament, and synovial space than in blood based on autoradiographic examination (Table 2). Furthermore, in the current study, drug-derived radioactivity determined by LSC was higher for bone and bone marrow than for plasma on day 14 (Table 1).

In conclusion, the high concentration of dalbavancin-associated radioactivity found in rabbit bone tissue over a prolonged period of time in this study, coupled with the once-a-week dosing regimen for dalbavancin in humans, suggests that dalbavancin may have the potential to treat bone and joint infection caused by gram-positive organisms. However, further investigation is warranted in animal models of osteomyelitis, followed by clinical safety and efficacy studies to assess the utility of dalbavancin for the treatment of bone and joint infection.

This study was supported by a grant from Vicuron Pharmaceuticals, a subsidiary of Pfizer Inc. Editorial support was funded by Pfizer Inc.

Editorial support was provided by Jean Turner at PAREXEL International.

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