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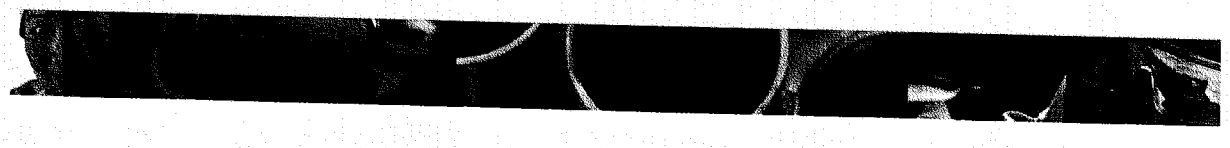


Daptomycin (Cidecin™) Treatment for Serious Gram-positive Infections Including Endocarditis

FP Tally, FB Oleson, CL Berman & MF DeBruin • Cubist Pharmaceuticals, Inc., Cambridge, MA, USA

INTRODUCTION

- Daptomycin is the first member of the class of cyclic lipopeptide antibiotics in clinical trials. It has a unique chemical structure and novel mechanism of action.
- Daptomycin has potent bactericidal activity against Gram-positive pathogens, including activity against resistant strains such as MRSA, VRE and GISA.
- Review of the initial Phase 2 studies indicates that daptomycin may be efficacious in the treatment of bacteremia and endocarditis:

Treatment Criteria	Bacteremia Favorable Response	Endocarditis Favorable Response
Clinical Symptoms		
Daptomycin*	17/19 (90%)	11/17 (65%)
Conventional therapy	1/2 (50%)	7/10 (70%)
Bacteriology		
Daptomycin*	17/19 (90%)	11/16 (69%)
Conventional therapy	1/2 (50%)	7/10 (70%)

*3 mg/kg q12h

- The daptomycin-treated endocarditis patients who failed to respond to treatment had lower mean trough serum concentrations ($p=0.02$) than those who had a favorable response. Success in the treatment of endocarditis may be even greater when patients receive higher doses of daptomycin.
- Based on these results, Cubist is continuing the evaluation of daptomycin in a worldwide clinical research program.
- Data presented herein are from a preliminary analysis of data from two ongoing Phase 2 clinical trials in 101 patients with bacteremia and serious Gram-positive infections, including patients with drug-resistant pathogens.

METHODS

CLINICAL TRIAL DESIGN

Two Multicenter, Open-Label, Phase 2A, Dose Selection Trials

- Bacteremia (Study BAC) – Initial Therapy
- Companion (Study RRC - Resistant, Refractory, Contra-indicated) – "Salvage" Therapy including bacteremia, cUTI, cSST, pneumonia and intra-abdominal infections

Inclusion Criteria

- Adults 18-85 years
- Clinical signs/symptoms of serious infection
- Gram-positive bacteremia or localized Gram-positive infection (RRC only)

Exclusion Criteria

- Shock
- Renal failure
- Neutropenia (<500 PMNs)
- AIDS (<200 CD4)
- Endocarditis, osteomyelitis, empyema, meningitis
- Prior effective antibiotic treatment

Treatment

- Patients were randomized to daptomycin 4 mg/kg q24h, 6 mg/kg q24h or 6 mg/kg loading dose followed by 3 mg/kg q12h
- Study BAC includes comparison to vancomycin 1 gm q12h or semi-synthetic penicillin 4-12 gm q12h (randomized)
- RRC patients with intra-abdominal infections, pneumonia or complicated skin/urinary tract infections received daptomycin 6 mg/kg or 4 mg/kg q24h, respectively (non-randomized)
- Duration is 7-14 days for BAC and 7-28 days for RRC

Endpoints

- Microbiological and clinical outcomes
- Safety assessments

RESULTS

DATA ANALYSIS GROUPS DEFINITIONS

- **Modified Intent-to-Treat Population (n=92)**
  All patients with documented Gram-positive bacterial infections who receive ≥ 1 dose of study medication
- **Clinically Evaluable Population (n=67)**
  Patients with documented Gram-positive infection who complete study evaluations that receive ≥ 4 days study treatment and who satisfy protocol eligibility and evaluation criteria
- **Microbiologically Evaluable Population (n=63)**
  Patients with appropriate bacteriologic cultures obtained in accordance with protocol sampling schemes (e.g., within 48 hours of initiating study therapy) and appropriate post-therapy evaluation
- **Safety Evaluable Population (n=101)**
  All patients who receive any amount of study drug

Table 1: Daptomycin Study Demographics

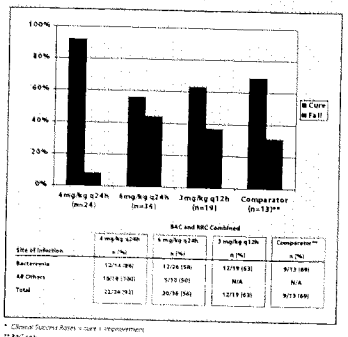
	Gender		Age	Race		
	Male	Female	Mean Years	Black	Caucasian	Other
BAC	50%	50%	59	23%	70%	7%
RRC	57%	43%	56	9%	85%	6%

Table 2: Daptomycin Study Completion/Withdrawal
Modified Intent-to-Treat

	4 mg/kg q24h BAC	4 mg/kg q24h RRC	6 mg/kg q24h BAC	6 mg/kg q24h RRC	3 mg/kg q12h BAC	3 mg/kg q12h RRC	Comparator BAC
Enrolled	12	14	20	18	16	6	13
Completed	10	11	14	11	12	3	9
Duration*	14	13	8	13	10	13	11
Discontinued	2	3	6	7	4	3	4
Patient decision	0	0	1	3	0	0	1
AE	2	0	1	2	1	1	0
Clinical failure	0	0	2	1	0	1	0
Death	0	1	1	1	1	1	1
Other	0	2	1	0	2	0	2

*Median days

Figure 1: Daptomycin Clinical Success Rates*
Total Modified Intent-to-Treat Population

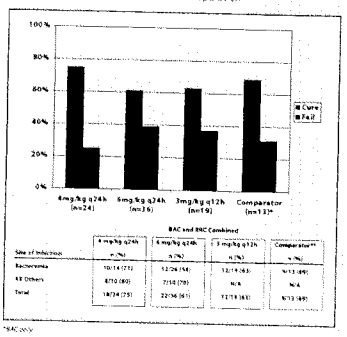


BAC and RRC Combined

Site of Infection	4 mg/kg q24h n (%)	6 mg/kg q24h n (%)	3 mg/kg q12h n (%)	Comparator** n (%)
Bacteremia	12/14 (86)	12/26 (58)	12/19 (63)	9/13 (69)
All Others	10/10 (100)	9/10 (90)	N/A	N/A
Total	22/24 (92)	20/36 (56)	12/19 (63)	9/13 (69)

* Clinical Success Rates = cure + improvement
** BAC only

Figure 2: Daptomycin Microbiologic Eradication Rates
Total Modified Intent-to-Treat Population



BAC and RRC Combined

Site of Infection	4 mg/kg q24h n (%)	6 mg/kg q24h n (%)	3 mg/kg q12h n (%)	Comparator** n (%)
Bacteremia	10/14 (71)	12/26 (58)	12/19 (63)	9/13 (69)
All Others	8/10 (80)	7/10 (70)	N/A	N/A
Total	18/24 (75)	20/36 (61)	12/19 (63)	9/13 (69)

*BAC only

Table 3: Daptomycin Clinical Success Rates*
BAC & RRC Protocols

Protocol	4 mg/kg q24h n (%)	6 mg/kg q24h n (%)	3 mg/kg q24h n (%)
BAC**	10/10 (100)	13/19 (68)	11/15 (73)
RRC***	12/14 (86)	7/17 (41)	1/4 (25)

*Total Modified Intent-to-Treat Population
** BAC = Bacteremia Study
*** RRC = Resistant, Refractory, Contra-indicated

Table 4: Adverse Events*
Safety Evaluable Population

	BAC and RRC Combined			
Body System	4 mg/kg q24h n=24 (%)	6 mg/kg q24h n=37 (%)	3 mg/kg q12h n=27 (%)	Comparator n=13 (%)
Patients with ≥ 1 Adverse Event	11 (47)	14 (41)	8 (40)	5 (45)
Cardiovascular	0	2 (6)	2 (10)	0
Gastrointestinal	2 (7)	7 (19)	2 (10)	3 (27)
Musculoskeletal	1 (4)	0	1 (5)	0
Nervous system	2 (7)	1 (3)	1 (5)	3 (27)
Skin & Subcutaneous Tissue	3 (13)	4 (11)	1 (5)	0

* Event by Investigator as Possibly or Probably Related to Study Drug Administration
** BAC Only

SERIOUS ADVERSE EVENTS*

- No serious adverse events at 4 mg/kg q24h
- One case of thrombocytopenia (33,000/cu mm at baseline) and one case of upper abdominal pain at 6 mg/kg q24h
- One case of AV block, one case of BUN/creatine increase and one case of leukopenia at 3 mg/kg q12h

* Assessed by Investigator as Possibly or Probably Related to Study Drug Administration

CONCLUSIONS

- Once-a-day daptomycin appears to be safe and effective in the treatment of bacteremia and serious Gram-positive infections
- Daptomycin is well tolerated with no trends in local and/or systemic adverse events
- Daptomycin demonstrated clinical activity in both susceptible and resistant Gram-positive bacteria
- The preliminary analysis of the clinical success rate at 4 mg/kg q24h in both the BAC (100%) and RRC (86%) studies appear to support the use of 4 mg/kg q24h for the treatment of bacteremia and other serious infections
- Based on this Phase 2 data with once-daily daptomycin and the previous studies in endocarditis, Cubist plans to begin investigating the efficacy and safety of once-daily daptomycin in patients with endocarditis

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In Vitro Activity of Daptomycin (Cidecin™) Against Contemporary Gram-positive Clinical Bacterial Isolates From 11 North American Medical Centers (NAMC)

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Abstract

Objectives: Daptomycin (DAP) is a lipopeptide which, in the presence of free calcium (Ca^{++}), is active against Gram-positive bacteria. DAP and vancomycin (VANC) were tested against 2803 clinical isolates gathered from NAMC. **Methods:** For broth microdilution tests, Mueller-Hinton broth contained NCCLS recommended Ca^{++} at 25 µg/mL and DAP was tested in a second broth adjusted to 50 µg/mL. **Results:** Overall, at 50 µg/mL Ca^{++}, DAP was 2 to 4 times more potent than when tested at 25 µg/mL. Against VANC-S and VANC-R enterococci, DAP was more effective and DAP was consistently more potent that VANC. MIC values (µg/mL) were:

Table 1.

Genera (#)	Drug	MIC_{50}	MIC_{90}	% Susceptible
Enterococcus (550)	DAP*	1	2	92.0
Enterococcus (550)	VANC	1	32	80.7
Staphylococcus (1094)	DAP*	0.25	0.5	100
Staphylococcus (1093)	VANC	1	2	100
Streptococcus (1099)	DAP*	0.12	0.25	99.7
Streptococcus (1099)	VANC	1	1	100
All others (50)	DAP*	0.12	2	91.5
All others (50)	VANC	1	2	95.7

*DAP MIC based on provisional breakpoints using NCCLS method not on clinical data.

Conclusion: DAP has been shown to be active in vitro against a variety of VANC-S and VANC-R strains of enterococci, as well as all species of staphylococci and streptococci.

Introduction

Daptomycin is a lipopeptide antibiotic with potent antimicrobial activity against Gram-positive bacteria. Its in vitro activity is influenced by the free calcium concentration in the medium. The present study was designed to:

- Assess the in vitro activity of daptomycin against contemporary clinical isolates of Gram-positive bacteria from multiple clinical centers
- Confirm the effect of Ca^{++} concentration on the in vitro activity against these isolates

Methods

Microorganisms:

- A total of 2803 Gram-positive bacterial isolates were provided by 11 North American centers (listed in Table 2) during the winter months of 1999. The number of isolates within each species or subgroup is provided in Table 3

Antimicrobial Agents:

- Daptomycin was provided as standardized powder by Cubist Pharmaceuticals, Inc.
- Penicillin and vancomycin were procured from commercial sources
- The concentrations of drugs tested were serial 2-fold dilutions ranging from 16 to 0.008 µg/mL for daptomycin, 16 to 1.0 µg/mL for vancomycin and 2.0 to 0.03 µg/mL for penicillin

Susceptibility Tests:

- MICs were determined by the broth microdilution test outlined by the NCCLS (M7-A4)
- Daptomycin was tested in Mueller-Hinton broth adjusted to contain either 25 or 50 µg/mL of free/ionic calcium Ca^{++}
- Penicillin and vancomycin were tested in Mueller-Hinton broth adjusted to contain 25 µg/mL of calcium
- When testing streptococci, the medium was supplemented with 2% to 3% lysed horse blood

Quality Control:

- On each day of testing, 1 or more of the following quality control organisms were tested: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Streptococcus pneumoniae* ATCC 49619
- Colony counts were performed on the inoculum suspensions of 2 randomly selected strains to assure appropriate inoculum density
- All MICs fell within the quality control ranges published by the NCCLS

Table 2. Participants in the Eleven Medical Centers Contributing Clinical Isolates

Lab #	Director/Supervisor	Laboratory	Location
1	Timothy Cleary, PhD	University of Miami	Miami, FL
2	Mary Jane Ferraro, PhD	Massachusetts General Hospital	Boston, MA
3	Dwight Hardy, PhD	University of Rochester Medical Center	Rochester, NY
4	Janet Hindler, MS	UCLA Medical Center	Los Angeles, CA
5	Stephen Jenkins, PhD	Carolinas Medical Center	Charlotte, NC
6	Gary Overturf, MD	Univ. of New Mexico Medical Center	Albuquerque, NM
7	Robert Rennie, PhD	University of Alberta Hospital	Edmonton, Alberta, Canada
8	Ken a, MD	University of Alabama at Birmingham	Birmingham, AL
9	Gary Procop, MD	The Cleveland Clinic Foundation	Cleveland, OH
10	Patrick Murray, PhD	Washington U. School of Medicine	St. Louis, MO
11	Mary Bauman, MT	Providence St. Vincent Medical Center	Portland, OR

Results

- Consistent with previous reports, the MICs of daptomycin determined in 50 µg/mL of Ca^{++} were significantly lower for all species than those tested in 25 µg/mL of Ca^{++}
- The overall distribution of daptomycin MICs in the 2 calcium concentrations is shown in Figure 1. The daptomycin MIC mode was 0.25 µg/mL when tested in 50 µg/mL of Ca^{++}, and 1.0 µg/mL when tested in 25 µg/mL of Ca^{++}
- The great majority of isolates had 2- to 4-fold higher MICs when tested in 25 µg/mL of Ca^{++} as compared to the same strains tested in 50 µg/mL of Ca^{++} (Figure 2). The same 2- to 4-fold difference in MICs can also be seen by comparing the geomean of each species (Table 3)
- Only 35 % of 550 strains of enterococci were susceptible to daptomycin when tested in 25 µg/mL of Ca^{++}, whereas 89% were susceptible when tested in 50 µg/mL of Ca^{++}
- Daptomycin appeared to be equally effective against vancomycin-resistant and vancomycin-susceptible strains of enterococci (Table 3)
- All staphylococci were susceptible to daptomycin and no appreciable difference in daptomycin MICs was observed between methicillin-resistant and methicillin susceptible strains (Table 3)
- Penicillin-susceptible, -intermediate, and -resistant isolates of *S. pneumoniae* all had essentially the same geometric mean daptomycin MICs (Table 3)

Figure 1. Daptomycin MIC Distribution in 25 vs 50 µg/mL Ca^{++}

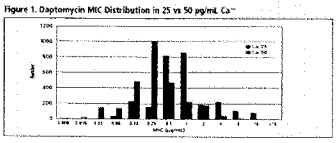


Figure 2. Daptomycin MICs with 25 vs 50 µg/mL of Ca^{++}

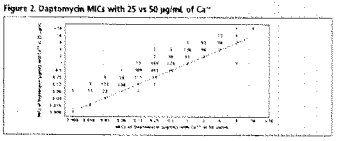


Table 3. Susceptibility of 2803 Gram-positive Clinical Isolates to Daptomycin and 2 Other Drugs*

Microorganism	Total No.	Antimicrobial Agent*	Range	MIC (µg/mL) 50%	90%	Geomean	Percentage Susceptible*
Bacillus spp.	8	Daptomycin-50	0.12 - 4.0	2.0		0.64	75.0
		Daptomycin-25	0.06 - 1.0	1.0		1.72	62.5
		Penicillin	0.06 - >2.0	1.0		0.76	NA
		Vancomycin	≤1.0 - 2.0	≤1.0		1.09	100
Corynebacterium jeikeium	7	Daptomycin-50	0.25 - 0.5	0.25		0.34	100
		Daptomycin-25	0.5 - 1.0	1.0		0.82	100
		Penicillin	>2.0	>2.0		>2.0	NA
		Vancomycin	≤1.0	≤1.0		≤1.0	100
Corynebacterium spp.*	22	Daptomycin-50	0.03 - 1.0	0.06	0.25	0.12	95.5
		Daptomycin-25	0.06 - >16	0.12	0.5	0.35	95.5
		Penicillin	≤0.03 - >2.0	0.5	>2.0	0.98	NA
		Vancomycin	≤1.0	≤1.0	≤1.0	≤1.0	100
Enterococcus avium	9	Daptomycin-50	0.25 - 4.0	1.0		1.08	77.8
		Daptomycin-25	1.0 - 16	4.0		3.43	44.4
		Penicillin	1.0 - >2.0	2.0		>2.0	-
		Vancomycin	≤1.0 - >16	≤1.0		1.71	88.9
Enterococcus faecalis Vancomycin Susceptible	377	Daptomycin-50	0.06 - 4.0	1.0	2.0	0.93	92.4
		Daptomycin-25	0.25 - 16	4.0	8.0	2.91	43.8
		Penicillin	0.06 - >2.0	2.0	>2.0	>2.0	-
		Vancomycin	≤1.0 - 4.0	≤1.0	2.0	1.27	100
Enterococcus faecalis Vancomycin Resistant	10	Daptomycin-50	0.12 - 4.0	2.0	4.0	1.42	80.0
		Daptomycin-25	0.25 - 8.0	8.0	8.0	4.60	10.0
		Penicillin	0.5 - >2.0	>2.0	>2.0	1.62	-
		Vancomycin	8.0 - >16	>16	>16	>16	0
Enterococcus faecium Vancomycin-Susceptible	90	Daptomycin-50	0.12 - 8.0	2.0	4.0	1.93	72.1
		Daptomycin-25	0.12 - >16	8.0	16	5.27	10.0
		Penicillin	0.5 - >2.0	>2.0	>2.0	>2.0	-
		Vancomycin	≤1.0 - 2.0	≤1.0	2.0	1.13	100
Enterococcus faecium Vancomycin-Resistant	90	Daptomycin-50	0.25 - 4.0	2.0	4.0	1.74	85.6
		Daptomycin-25	0.5 - 16	4.0	8.0	4.56	14.4
		Penicillin	0.12 - >2.0	>2.0	>2.0	>2.0	-
		Vancomycin	8.0 - >16	>16	>16	>16	0
Enterococcus gallinarum	8	Daptomycin-50	1.0 - 8.0	1.0		1.83	75.0
		Daptomycin-25	2.0 - 16	4.0		4.36	25.0
		Penicillin	1.0 - >2.0	>2.0		>2.0	-
		Vancomycin	≤1.0 - >16	4.0		8.00	50.0
Enterococcus spp.*	6	Daptomycin-50	0.03 - 4.0	1.0		0.79	83.3
		Daptomycin-25	0.03 - 8.0	2.0		1.25	66.7
		Penicillin	0.12 - 2.0	0.5		0.56	100
		Vancomycin	≤1.0 - 16	≤1.0		2.25	83.3
Lactobacillus spp.	10	Daptomycin-50	≤0.008 - 8.0	0.5	4.0	0.41	80.0
		Daptomycin-25	≤0.008 - 16	0.5	8.0	0.79	60.0
		Penicillin	≤0.03 - 1.0	≤0.03	1.0	0.07	NA
		Vancomycin	≤1.0 - >16	≤1.0	>16	2.30	80.0
Micrococcus spp.	13	Daptomycin-50	0.03 - 0.25	0.06	0.12	0.06	100
		Daptomycin-25	0.06 - 0.5	0.12	0.25	0.14	100
		Penicillin	≤0.03 - >2.0	0.12	0.12	0.12	92.3
		Vancomycin	≤1.0 - 2.0	≤1.0	≤1.0	1.04	100
Staphylococcus aureus Methicillin-Susceptible	375	Daptomycin-50	0.03 - 1.0	0.25	0.5	0.29	100
		Daptomycin-25	0.12 - 4.0	1.0	1.0	0.82	99.7
		Penicillin	≤0.03 - >2.0	≤0.03	>2.0	>2.0	12.0
		Vancomycin	≤1.0 - 2.0	≤1.0	≤1.0	1.01	100
Staphylococcus aureus Methicillin-Resistant	172	Daptomycin-50	0.12 - 2.0	0.25	0.5	0.31	100
		Daptomycin-25	0.5 - 8.0	1.0	1.0	0.96	99.4
		Penicillin	0.25 - >2.0	>2.0	>2.0	>2.0	0
		Vancomycin	≤1.0 - 2.0	≤1.0	≤1.0	1.04	100
Staphylococcus epidermidis	70	Daptomycin-50	0.12 - 1.0	0.25	0.5	0.30	100
		Daptomycin-25	0.25 - 2.0	1.0	2.0	0.81	100
		Penicillin	≤0.03 - >2.0	>2.0	>2.0	>2.0	5.0
		Vancomycin	≤1.0 - 2.0	2.0	2.0	1.53	100
Staphylococcus haemolyticus	5	Daptomycin-50	0.12 - 0.25	0.25		0.22	100
		Daptomycin-25	0.25 - 1.0	0.5		0.57	100
		Penicillin	≤0.03 - >2.0	>2.0		1.31	20.0
		Vancomycin	≤1.0 - 2.0	≤1.0		1.15	100
Staphylococcus saprophyticus	6	Daptomycin-50	0.25 - 0.5	0.25		0.32	100
		Daptomycin-25	0.25 - 1.0	0.5		0.56	100
		Penicillin	≤0.03 - >2.0	0.06		0.39	50.0
		Vancomycin	≤1.0 - 2.0	≤1.0		1.26	100
Staphylococcus Other species	5	Daptomycin-50	0.12 - 0.5	0.25		0.25	100
		Daptomycin-25	0.25 - 1.0	1.0		0.66	100
		Penicillin	≤0.03 - 2.0	0.25		0.50	20.0
		Vancomycin	≤1.0	≤1.0		≤1.0	100
Staphylococcus spp. NOS Coagulase-Negative Methicillin-Susceptible	174	Daptomycin-50	0.016 - 2.0	0.25	0.5	0.28	100
		Daptomycin-25	0.016 - 4.0	1.0	1.0	0.62	99.4
		Penicillin	≤0.03 - >2.0	0.5	2.0	0.27	38.5
		Vancomycin	≤1.0 - 4.0	≤1.0	2.0	1.18	100
Staphylococcus spp. NOS Coagulase-Negative Methicillin-Resistant	334	Daptomycin-50	0.12 - 2.0	0.25	0.5	0.31	100
		Daptomycin-25	0.25 - 8.0	1.0	1.0	0.81	99.7
		Penicillin	≤0.03 - >2.0	>2.0	>2.0	>2.0	0
		Vancomycin	≤1.0 - 4.0	2.0	2.0	1.45	100

Table 3 Continued on back

A-19

Pharmacodynamics of Daptomycin (D) Against Vancomycin-Resistant *Enterococcus faecium* (VREF) and Methicillin-Resistant *Staphylococcus aureus* (MRSA) in an In Vitro Infection Model With Simulated Endocardial Vegetations (SEVs).

Ronda L. Akins[1,2] and Micael J. Rybak[1,2] • The Anti-Infective Research Laboratory, Detroit Receiving Hospital[1] and Wayne State University,[2] Detroit, MI, USA

Abstract

Objectives: Daptomycin (D) is an investigational lipopeptide antibiotic active against gram-positive organisms, including MRSA and VRE, with a unique mechanism of action resulting in interference with cell membrane transport. We evaluated the activity of D versus Vancomycin (V) against MRSA and D against VREF in an in vitro SEV model.

Methods: SEV models were infected with ~10^8 CFU/g of MRSA-494 or VREF-R590. D was dosed to achieve peaks/troughs of 140/17.5 or 80/10 µg/ml based on a regimen of 10 (D10) mg/kg or 6 (D6) mg/kg q 24h with a simulated half-life of 8h. V was dosed to achieve standard concentrations and a half-life of 6h was simulated. All models contained clots with an average total protein 6.8-7.4 g/dL and albumin 3.0-3.5 g/dL. SEV bacterial density (CFU/g) was determined at time = 0, 8, 24, 32, 48 and 72h.

Results: MICs/MBCs for D was 0.25/0.25 mg/L for MRSA-494. 2/2 mg/L for VREF-590 and V was 0.5/1 mg/L for MRSA-494. During the first 8h D reduced the initial inoculum by an average of 4.4 (D6)/6.5 (D10) and 3.4 (D6)/5.1 (D10) logs CFU/g for MRSA and VREF, respectively and with V against MRSA there was a 3 log reduction by 72h. Concentration-dependent killing was observed against VREF with D10 achieving a 1.7 log greater kill than D6 at 8h. Against MRSA D has a 5-5.6 log reduction at 72h with minimal regrowth (<1 log CFU/g). V had more static activity with only a 1.5 log reduction at 72h for MRSA. Against VREF D10 maintained 99.9% kill during the entire 72h period. While D6 maintained 99.9% kill for 72h regrowth was noted after 48h. D Peak/MIC and AUC/MIC (in absence/presence of albumin) was 452-570/113-143 and 4233-6020/1010-1505 for MRSA and 50-65/2-3 and 497-710/31-44 for VREF.

Conclusions: D demonstrated significant activity against VREF and against MRSA compared to V ($p \leq 0.05$).

Background

- Multi-drug resistant gram-positive infections have been steadily increasing worldwide. As with most drug-resistant organisms, treatment options are limited. Therefore, the need to explore new therapeutic alternatives are necessary.
- Daptomycin is one of these potential alternatives. It is a lipopeptide antibiotic that is derived from *Streptomyces roseosporus*, with broad activity against gram-positive organisms. Recently licensed by Cubist Pharmaceuticals, daptomycin is currently being evaluated with newly designed dosing regimens.

Objectives

- To evaluate the activity of Daptomycin against Vancomycin-Resistant *Enterococcus faecium* and Daptomycin vs Vancomycin against Methicillin-Resistant *Staphylococcus aureus* in an in vitro simulated endocardial vegetation model.

Materials and Methods

Organism

- Clinical isolates of VREF-590 and MRSA-494 was used in the in vitro SEV model.

Antimicrobial Agent

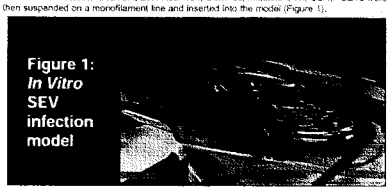
- Daptomycin (Lot # 44BYO) was obtained from Cubist Pharmaceuticals, Inc., Cambridge, Massachusetts, USA.
- Vancomycin (Lot # INJ03M) was commercially purchased from Sigma Chemical Company, St. Louis, Missouri).

Medium

- Mueller-Hinton broth (Difco, Detroit, USA) supplemented with calcium (75 mg/L) for D and calcium (25 mg/L) for V and magnesium (12.5 mg/L) for both (SMHB) was used for microdilution susceptibility testing and in the in vitro SEV model. Susceptibility testing was performed in absence and presence of albumin (3.5-4 g/dL). Models protein content was attributable to components of the SEVs.

In Vitro SEV Model

- SEVs were prepared by combining human platelets (0.025 mL, providing ~250,000-500,000 per SEV), human cryoprecipitate antihemolytic factor (0.4 mL American National Red Cross, Detroit, MI, USA), 0.1 ml of organism suspension (Initial inoculum ~10^8 CFU/g) and 0.05 ml of a 5000U/ml solution thrombin, Lot #RJ39A164, GenTrac, Middleton, WI, USA). SEVs were then suspended on a monofilament line and inserted into the model (Figure 1).



- Antibiotics were administered into the central compartment of a 250 ml glass model at doses to simulate D 10 or 6 mg/kg q24h and V 1g q12h achieving peak/trough serum concentrations of approximately 140/17.5 or 80/10µg/ml and 30-35/5-10 µg/ml, respectively. Fresh SMHB was pumped into the central compartment, continuously mixed by a stirrer bar, displacing antibiotic-containing media at a rate equal to projected half-lives of 8h for D and 6h for V.
- Three intact SEVs were removed and homogenized from each model (total of 6) at time=0,8,24,48 and 72h to determine change in bacterial density. Samples were diluted in normal saline accordingly and plated on Tryptic Soy Agar (TSA) plates and incubated for 24 hours at 37°C prior to bacterial count
- Microbioassays were performed to determine actual antibiotic concentrations achieved in the model. Between day CV% of standards (150, 100, 10 µg/ml) ≤ 10% with a lower limit of detection = 1.25 µg/ml.

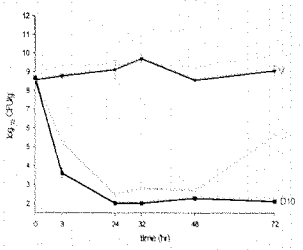
Resistance

- 100 µl of the 72h samples were plated onto 4-8x MIC antibiotic plates. Plates were incubated for 48 hours at 37°C to evaluate for emergence of resistance.

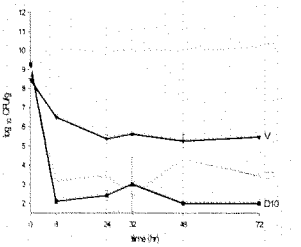
Statistics

- Change in $\log_{10}$ CFU/ml from 0 to 72h was compared using ANOVA with Tukey's Post Hoc test. Time to achieve 99.9% killing was determined by linear regression. A *P* value ≤ 0.05 was considered statistically significant.

Figure 2A



2B



- D MICs (in absence/presence of albumin) for MRSA-494 and VREF-590 was 0.25/1 and 2/32 mg/L, respectively. V MICs for MRSA and VREF were 0.5 mg/L and >64mg/L, respectively.
- 99.9% kill was achieved by 8h for all D regimens vs both VREF and MRSA, however slight regrowth occurred by 72h for D6 vs VREF. (Figure 2A, 2B)
- D achieved significant kill ($p \leq 0.05$) against VREF and MRSA with a 3 log decrease still evident at 72h.
- D's Peak/MIC and AUC/MIC (in absence/presence of albumin) for VREF-590and MRSA-494 with D10 were 64.9/2.0, 570.4/142.8 and 710.2/44.4, 6020.4/1505.1 and D6 were 50.0/3.1, 452.4/113.1 and 496.8/31.1, 4038.8/1009.7, respectively.
- V's Peak/MIC and AUC/MIC were 0.6, 78.4 and 7.1, 1135.2, respectively, for VREF-590 and MRSA-494.
- No evidence of resistance was found throughout the experimental period for any of the regimens.

Bacterial Counts ($\log_{10}$ CFU/g)
Average ± S.D.

Organism	Regimen	Initial (0h)	Final (72h)
VREF (R590)	Growth Control	8.77 ± 0.01	9.85 ± 0
	D 10mg/kg/d	8.66 ± 0.06	2.1 ± 0.08*
	D 6mg/kg/d	8.62 ± 0.08	5.57 ± 1.07*
	V 1g q12h	8.55 ± 0.01	9.02 ± 0.25
MRSA-494	Growth Control	8.92 ± 0.04	10.14 ± 0.17
	D 10mg/kg/d	9.28 ± 0.16	2.0 ± 0*
	D 6mg/kg/d	8.95 ± 0.07	3.41 ± 1.19*
	V 1g q12h	8.45 ± 0.14	5.45 ± 0.07*

* $p \leq 0.05$

- Daptomycin demonstrates significant ($p \leq 0.05$) activity against vancomycin resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus*. Dramatic kill (99.9%) by 8h was noted for both daptomycin regimens against VREF and MRSA compared to vancomycin ($p \leq 0.05$).
- No resistance developed against either organism with any of the regimens.
- Further study of daptomycin against these and other resistant organisms is warranted to better characterize the activity and pharmacodynamics properties of this agent

#1771

Activity of Daptomycin (D), Arbekacin (A), Vancomycin (V) and Gentamicin (G) Against Two Clinical Strains of Vancomycin-Intermediate Resistant *Staphylococcus aureus* (VISA) in an In Vitro Pharmacodynamic Infection Model (IVPM)

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Abstract

Daptomycin (D), a lipopeptide, and arbekacin (A), a unique aminoglycoside, possess significant activity against resistant gram-positive organisms. We evaluated their activity and vancomycin and gentamicin against 2 VISA (Mu-50 and HIP5836 [992]) isolates and a control strain (MRSA-67). An IVPM, initial inoculum of ~10^6 CFU/ml, was utilized over 48h and sampled to determine organism density. D was dosed 6 (D6) or 4 (D4) mg/kg q24h or 3 (D3) mg/kg q12h for estimated free peak (PK)/trough (TR) concentrations of 6/0.75, 4/0.5 and 3/1.1 mg/L, respectively, and a simulated half-life of 8h. A was dosed q12h for PK/TR of 8/0.5 mg/L with a simulated half-life of 3h. V and G were dosed to achieve standard concentrations. D MIC/MBC were 0.5/1 mg/L for Mu-50 and HIP5836 [992] and 0.125/0.5 mg/L for MRSA-67. A MIC/MBC was 2/8 mg/L for Mu-50, 0.125/0.5 mg/L for HIP5836 [992] and 0.125/0.25 mg/L for MRSA-67. Against Mu-50 all D regimens resulted in time to 99.9% kill (T99) by 6h, although regrowth was noted at 48h. Minimal kill, not resulting in T99, occurred for A with significant regrowth at 48h. V resulted in static activity; G was equal to growth control. D6+A resulted in additive activity at 48h. Against HIP5836 [992] all D regimens resulted in T99 by 6h. However, D4 was noted to have significant regrowth by 48h. D6 and D3 had ≤0.5 log CFU/ml regrowth. V demonstrated static activity. Against MRSA-67 bactericidal activity was achieved for all regimens. No resistance developed for any regimen. D AUC/MIC and PK/MIC were 79-116/320-461 and 6-12/24-48 for VISA/MRSA, respectively. D alone or with A demonstrated significant activity against VISA and MRSA ($p \leq 0.05$).

Background

- Multi-drug resistant gram-positive infections have been steadily increasing. VISA has been isolated in Japan, the United States, France and Hong Kong with 8 strains now documented. As with most drug-resistant organisms, treatment options are limited. Therefore, the need to explore new therapeutic alternatives is necessary.
- Daptomycin is an investigational lipopeptide antibiotic that is derived from *Streptomyces roseosporus* with broad activity against gram-positive organisms. Clinical studies in the late 1980s were stopped due to less than desirable outcomes using earlier dosing regimens of 2-3 mg/kg q12 hours. The high protein binding (approximately 93%) of this drug is one possible explanation to these outcomes. Recently re-licensed by Cubist Pharmaceuticals, daptomycin is currently being evaluated with higher and/or redesigned dosing regimens.

Objective

- To evaluate and compare the activity of daptomycin, arbekacin, vancomycin and gentamicin alone or in combination against two clinical isolates of VISA in an in vitro pharmacodynamic infection model.

Materials and Methods

Bacterial strains.

- Two VISA strains were evaluated: Mu-50 (Juntendo Hospital, Tokyo, Japan) and HIP5836 [992] (New Jersey strain, Centers for Disease Control, Atlanta, Georgia). MRSA-67 was also tested as a clinical control strain.

Antibiotics.

- Daptomycin (lot 444BY0, Cubist Pharmaceuticals Inc., Cambridge, Massachusetts) and arbekacin (lot ABKMC-1300, Meiji Seika Kaisha, Ltd., Pharmaceutical Division, Tokyo, Japan) were used. Vancomycin (lot 1NJ03M, Sigma Chemical Company, St. Louis, Missouri) and gentamicin (lot 96H0975, Sigma Chemical Company, St. Louis, Missouri) were commercially purchased.

Medium.

- Mueller-Hinton Broth (Difco Laboratories) supplemented with calcium (25 mg/L), magnesium (12.5 mg/L) (SMHB) was used for all antibiotics, except daptomycin, for susceptibility testing and in vitro models. Daptomycin was tested in SMHB supplemented with calcium (75 mg/L) and magnesium (12.5 mg/L).

Susceptibility testing.

- MICs and MBCs of the antibiotics were determined by broth microdilution in SMHB according to guidelines of the National Committee for Clinical Laboratory Standards (NCCLS).

In vitro infection model.

- One-compartment in vitro infection model (250ml) allowing for simulation of pharmacokinetics of drugs in human was utilized.
- Antibiotics were administered as boluses into the central compartment to achieve targeted peak serum concentrations (PK). Fresh media was continuously supplied and removed from the compartment along with the drug via a peristaltic pump set to achieve the half-lives ($T_{1/2}$) of the antibiotics. Samples were removed over a 48h period and were plated onto Tryptic Soy Agar (TSA) and incubated at 37°C for 24h to determine colony counts.
- Antibiotic regimens included: Daptomycin 6 or 4 mg/kg every 24h and 3 mg/kg every 12h for simulated free PK/TR concentrations of 6/0.75, 4/0.5 and 3/1.1 mg/L with a half-life of 8h. Arbekacin 100 mg q12h for a PK/TR of 8/0.5 mg/L with a half-life of 3h. Vancomycin for a PK/TR of 30/10 mg/L with a half-life of 6h. Gentamicin for a PK/TR of 5/0.5 with a half-life of 3h.
- Duplicate samples (1.0ml) were obtained from the central compartment of each model to determine antibiotic concentrations. Concentrations of daptomycin were determined by microbioassay utilizing *Micrococcus luteus* ATCC 9341. All other antibiotic concentrations were determined utilizing fluorescence polarization immunoassay (TDx - Abbott Laboratories, Irving, Texas). The half-lives, AUC and peak concentrations of the antibiotics were determined by trapezoidal methods utilizing RStrip software, version 3.1 (Micromath, Salt Lake City, UT).

Statistics.

- Time to 99.9% kill was determined by linear regression.
- Changes in $\log_{10}$ CFU/ml with respect to AUC/MIC and peak/MIC at 48 hours were compared using ANOVA with Tukey's Post-Hoc test. Regression analysis was used to determine correlation between the parameters.
- P values ≤0.05 were considered statistically significant.

Results

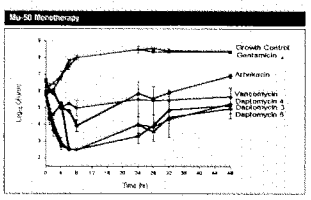
Table 1. Antibiotic Susceptibility (MIC/MBC mcg/ml)

	Mu-50	HIP5836 [992]	MRSA-67
Daptomycin	0.5/1.0	0.5/1.0	0.125/0.5
Arbekacin	2.0/8.0	0.125/0.5	0.125/0.25
Vancomycin	8.0/8.0	8.0/8.0	0.5/1.0
Gentamicin	128/128	0.5/1.0	0.25/0.5

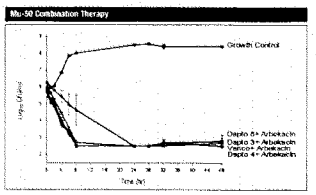
Table 2. Average Pharmacokinetic Parameters

	AUC$_{24}$/MIC			Peak/MIC		
	Mu-50	HIP5836 [992]	MRSA-67	Mu-50	HIP5836 [992]	MRSA-67
Dapto 6	116±7.6	112±9.3	461±30.2	11.7±0.6	11.6±0.7	46±2.8
Dapto 4	80±3.7	80±3.6	320±14.9	7.7±0.4	7.5±0.4	30.4±1.6
Dapto 3	93±4.1	96±3.7	371±15.4	6±0.3	6.3±0.4	24.2±1.2
Arb	26±1.8	521±28.0	520±28.8	4±0.1	82.4±1.4	65.4±1.6
Vanco	53±0.4	52±0.5	834±7.2	3.7±0.3	4.4±0.4	68.4±5.4
Gent	0.4±0.01	96±2.8	201±5.7	0.04±0.0003	10.7±0.08	21.6±0.15

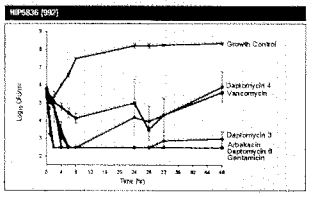
Mu-50 Monotherapy



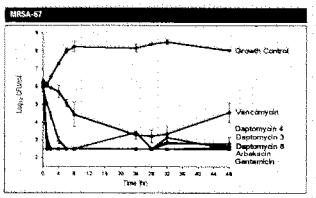
Mu-50 Combination Therapy



HIP5836 [992]



MRSA-67



- By 6 hours all daptomycin regimens resulted in 99.9% kill (≥3 $\log_{10}$ reduction in CFU/ml) against all isolates. However, regrowth was noted against Mu-50 with all dosages and with daptomycin 4 mg/kg every 24h for HIP5830 [992].
- Arbekacin alone resulted in minimal kill and significant regrowth against Mu-50. Against HIP5836 [992] and MRSA-67 99.9% kill was achieved within 2 hours and maintained throughout the 48 hour experimental period.
- Combination therapy with daptomycin (at all regimens) + arbekacin against Mu-50 resulted in significant kill (99.9%) by 8 hours, which remained at the limit of detection over 48 hours.
- Vancomycin resulted in static activity against the 2 VISA strains and minimal kill against MRSA-67. However, synergistic activity with arbekacin against Mu-50 was achieved.
- Gentamicin produced significant activity against HIP5836 [992] and MRSA-67. However, Mu-50 was fully resistant to gentamicin, resulting in no kill.
- AUC/MIC ratio for daptomycin ranged from 79-461, with daptomycin 6 having the highest ratio against all strains, followed by daptomycin 3 and daptomycin 4. Arbekacin demonstrated similar ratios for HIP5836 [992] and MRSA-67, but a considerably lower ratio for Mu-50.

Conclusions

- **Daptomycin demonstrated significant activity against these two VISA isolates ($p < 0.05$).**
- **Combination therapy with daptomycin and arbekacin produced synergistic activity against Mu-50.**
- **Daptomycin 4 mg/kg every 24h against HIP5836 [992] resulted in significant bacterial regrowth at 48 hours ($p \leq 0.05$), possibly due to a total dose phenomena effect, since daptomycin 3 mg/kg every 12 hours or daptomycin 6 mg/kg every 24 hours resulted in equal and significant kill.**
- **There appeared to be a trend toward AUC/MIC and PK/MIC association with decreased CFU/ml at 24 and 48 hours. However, further pharmacodynamic parameters need to be examined using a wider variety of organisms with differing MICs.**

Akins and Rybak, ICAAC 1999, Poster #1771

#350

Daptomycin Susceptibility Tests: Provisional Criteria, Quality Control, and Importance of Ca^{++} Concentration in Test Media

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Abstract

For 513 Gram-positive isolates (including 51 MRSA and 48 VRE), MICs of daptomycin varied inversely with the Ca^{++} concentration in the broth (25, 50, and 75 µg/ml). In 6 lots of Mueller-Hinton agar, the total Ca^{++} levels were 28, 32, 6, 28, 32, and 36 µg/ml. Daptomycin zone diameters on the lot with 6 µg/ml of Ca^{++} were 6 mm smaller than on the other lots. In Mueller-Hinton broth, changing Ca^{++} from 25 to 50 µg/ml changed few interpretations except for tests of enterococci; at 25 µg/ml, many were resistant or intermediate, at 50 µg/ml, most were susceptible (MIC ≤2.0 µg/ml). Since early clinical trials suggest that enterococci may respond to daptomycin therapy, physiologic concentrations of free Ca^{++} in serum (45-55 µg/ml) are recommended for Mueller-Hinton broth when testing daptomycin. For 30 µg disk tests and MICs in broth with 50 µg/ml of Ca^{++}, the following provisional breakpoints are proposed for daptomycin: Susceptible = ≥16 mm (MIC ≤2 µg/ml) and Resistant = ≤12 mm (MIC ≥8 µg/ml). Because of lot-to-lot variation of Ca^{++} levels, each new lot of Mueller-Hinton agar must be screened with QC strains. Proposed daptomycin MIC QC ranges: *S. aureus* ATCC 29213 – 0.25 to 1.0 µg/ml; *E. faecalis* ATCC 29212 – 1.0 to 4.0 µg/ml; disk test QC range: *S. aureus* ATCC 25923 – 18 to 23 mm; for *S. pneumoniae* ATCC 49619, MIC 0.06 to 0.5 µg/ml and zones 19 to 28 mm.

Introduction

Daptomycin is a lipopeptide antibiotic with potent antimicrobial activity against Gram-positive bacteria, including MRSA and VRE. Its MICs have previously been shown to be affected by the Ca^{++} concentration in the test medium.

The present studies were designed to:

1. Determine the appropriate Ca^{++} concentration for *in vitro* daptomycin susceptibility tests.
2. Compare the *in vitro* activity of daptomycin with that of vancomycin and teicoplanin.
3. Assess disk diffusion test interpretive criteria for daptomycin.
4. Determine the daptomycin QC ranges for four standard Gram-positive QC strains.

Methods

1. A total of 513 Gram-positive bacteria (listed in Table 1) were tested for susceptibility to daptomycin, vancomycin, and teicoplanin by the NCCLS broth microdilution method (M7-A4). In addition to standard cation-adjusted Mueller-Hinton broth (25 µg/ml of Ca^{++}), daptomycin MICs were also determined in Mueller-Hinton broth containing 50 and 75 µg/ml of Ca^{++}.
2. The staphylococci and enterococci tested above and 156 pneumococci were tested for susceptibility to daptomycin by broth microdilution as well as by the NCCLS disk diffusion method (M2-A6) using two lots of Mueller-Hinton agar, one containing 28 µg/ml of Ca^{++} and the other containing 6 µg/ml of Ca^{++}.
3. Ten laboratories participated in the study to determine QC ranges for four QC strains. The procedure followed the guidelines recommended by the NCCLS (M23-A). In each laboratory five different lots of Mueller-Hinton broth (for MIC ranges) and three lots of Mueller-Hinton agar, as well as two lots of daptomycin 30 µg disks (for disk diffusion zone diameter ranges), were used on each of 10 test days. This resulted in 500 MIC determinations for each organism tested, and a total of 900 zone diameter measurements for each organism tested.

Results

Table 1: In Vitro Activity of Daptomycin in Broth with 3 Calcium Concentrations vs. Vancomycin and Teicoplanin in CAMHB

Microorganism (No. tested)	Antimicrobial agent	Range	MIC (µg/ml) in broth containing 25 µg/ml Ca^{++}: 50%	90%	% Tested
Staphylococcus aureus (51) Methicillin-Resistant	Daptomycin (S)	0.5 - 1.0	1.0	1.0	100
	Daptomycin (I)	0.25 - 1.0	0.5	1.0	100
	Daptomycin (R)	0.06 - 0.5	0.25	0.5	100
	Vancomycin	0.5 - 1.0	0.5	1.0	100
	Teicoplanin	0.5 - 2.0	1.0	2.0	100
Staphylococcus aureus (97) Methicillin-Susceptible	Daptomycin (S)	0.12 - 1.0	0.5	1.0	100
	Daptomycin (I)	0.12 - 1.0	0.5	1.0	100
	Daptomycin (R)	0.06 - 0.5	0.25	0.5	100
	Vancomycin	0.5 - 2.0	1.0	1.0	100
	Teicoplanin	0.5 - 2.0	1.0	1.0	100
Staphylococcus epidermidis (51) Methicillin-Resistant	Daptomycin (S)	0.06 - 0.5	0.5		100
	Daptomycin (I)	0.06 - 0.5	0.5		100
	Daptomycin (R)	0.06 - 0.5	0.5		100
	Vancomycin	0.5 - 1.0	1.0		100
	Teicoplanin	0.5 - 1.0	1.0		100
Staphylococcus epidermidis (76) Methicillin-Susceptible	Daptomycin (S)	0.12 - 0.5	0.5	0.5	100
	Daptomycin (I)	0.12 - 0.5	0.5	0.5	100
	Daptomycin (R)	0.06 - 0.5	0.25	0.5	100
	Vancomycin	0.5 - 1.0	0.5	1.0	100
	Teicoplanin	0.5 - 1.0	0.5	1.0	100
Staphylococcus hominis (20)	Daptomycin (S)	0.12 - 0.5	0.5	0.5	100
	Daptomycin (I)	0.12 - 0.5	0.5	0.5	100
	Daptomycin (R)	0.06 - 0.5	0.25	0.5	100
	Vancomycin	0.5 - 1.0	0.5	1.0	100
	Teicoplanin	0.5 - 1.0	0.5	1.0	100
Coag. neg. staphylococci (28) Not further identified Methicillin-Susceptible	Daptomycin (S)	0.25 - 1.0	0.5	1.0	100
	Daptomycin (I)	0.25 - 1.0	0.5	1.0	100
	Daptomycin (R)	0.12 - 0.5	0.25	0.5	100
	Vancomycin	1.0 - 2.0	1.0	2.0	100
	Teicoplanin	0.5 - 2.0	1.0	2.0	100
Coag. neg. staphylococci (9) Not further identified Methicillin-Resistant	Daptomycin (S)	0.5 - 1.0	0.5		100
	Daptomycin (I)	0.5 - 1.0	0.5		100
	Daptomycin (R)	0.25 - 0.5	0.5		100
	Vancomycin	1.0 - 2.0	2.0		100
	Teicoplanin	0.5 - 2.0	1.0		100
Enterococcus faecalis (51) Vancomycin-Susceptible	Daptomycin (S)	1.0 - 4.0	2.0	2.0	100
	Daptomycin (I)	1.0 - 4.0	2.0	2.0	100
	Daptomycin (R)	0.5 - 2.0	1.0	2.0	100
	Vancomycin	1.0 - 4.0	2.0	2.0	100
	Teicoplanin	0.5 - 2.0	1.0	1.0	100
Enterococcus faecium (50) Vancomycin-Resistant	Daptomycin (S)	1.0 - 4.0	2.0	2.0	100
	Daptomycin (I)	1.0 - 4.0	2.0	2.0	100
	Daptomycin (R)	0.5 - 2.0	1.0	2.0	100
	Vancomycin	>256 - >256	>256	>256	0
	Teicoplanin	>256 - >256	>256	>256	0
Enterococcus faecium (50) Vancomycin-Susceptible	Daptomycin (S)	1.0 - 4.0	2.0	2.0	100
	Daptomycin (I)	1.0 - 4.0	2.0	2.0	100
	Daptomycin (R)	0.5 - 2.0	1.0	2.0	100
	Vancomycin	0.5 - 1.0	0.5	1.0	100
	Teicoplanin	0.5 - 1.0	0.5	1.0	100
Group D streptococci (20)	Daptomycin (S)	0.12 - 0.5	0.5	0.5	100
	Daptomycin (I)	0.12 - 0.5	0.5	0.5	100
	Daptomycin (R)	0.06 - 0.5	0.25	0.5	100
	Vancomycin	0.12 - 0.5	0.25	0.5	100
	Teicoplanin	0.06 - 0.5	0.12	0.5	100
Viridans streptococci (20)	Daptomycin (S)	0.06 - 0.5	0.5	0.5	100
	Daptomycin (I)	0.06 - 0.5	0.5	0.5	100
	Daptomycin (R)	0.03 - 0.25	0.12	0.25	100
	Vancomycin	0.5 - 1.0	0.5	1.0	100
	Teicoplanin	0.5 - 1.0	0.5	1.0	100

Numbers following organisms indicate Ca^{++} concentration in mg/L.

Figure 1: Daptomycin MICs for Staphylococci (n=101) and Enterococci (n=98) with 50 vs. 25 mg/L of Ca^{++}

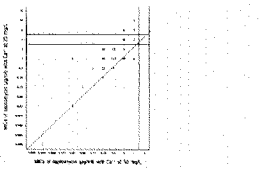

Table 2: Effect of Ca^{++} Content of M-H Agar on Daptomycin Zone Diameters

M-H Agar Lot	pH	Ca^{++} Content	Daptomycin vs. *S. aureus* ATCC 25923
A	7.15	28 µg/ml	20 mm
B	7.33	28 µg/ml	20 mm
C	7.30	35 µg/ml	21 mm
D	7.26	32 µg/ml	20 mm
E	7.28	32 µg/ml	20 mm
F	7.22	6 µg/ml	14 mm
QC Range			18-23 mm

Figure 2: Daptomycin Zone Diameters on Two Lots of Mueller-Hinton Agar

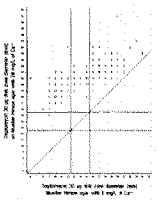

Figure 3: Daptomycin Disk Test vs. MICs with 50 mg Ca^{++} per Liter (all organisms)

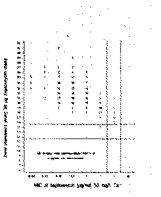

Table 3: Proposed Daptomycin QC Ranges

QC Strain	Proposed Range	% in Range
	MICs	
S. aureus ATCC 29213	0.25 - 1.0 µg/ml	95.3
E. faecalis ATCC 29212	1.0 - 4.0 µg/ml	96.7
S. pneumoniae ATCC 49619	0.06 - 0.5 µg/ml	99.7
	DISK TESTS	
S. aureus ATCC 25923	18 - 23 mm	98.8
S. pneumoniae ATCC 49619	19 - 28 mm	99.5

Results and Discussion

A. Effect of Ca^{++} concentration on daptomycin MIC tests:

Table 1 summarizes the *in vitro* activity of daptomycin in three different Ca^{++} concentrations. This is also graphically shown in Figure 1. Against all species daptomycin was significantly active in 50 than in 25 µg/ml of Ca^{++}. Tests in 75 µg/ml of Ca^{++} added little to daptomycin activity. Assuming a MIC susceptible breakpoint for daptomycin of ≤2.0 µg/ml, the Ca^{++} concentration had little effect on interpretive results except for enterococci. Many of these were not susceptible in the lower concentration. For this reason, and since 50 µg/ml is the normal concentration of Ca^{++} in serum, it appears reasonable to determine daptomycin MICs at this concentration.

B. Daptomycin compared to vancomycin and teicoplanin:

These data are summarized in Table 1. Daptomycin was the most active of the three drugs against staphylococci, including MRSA. 100% were susceptible to ≤2.0 µg/ml of daptomycin. Daptomycin was also the most active agent against VRE, while teicoplanin was most active against vancomycin-susceptible enterococci. All streptococcal species were highly susceptible to all three drugs. Daptomycin had poor activity against *L. monocytogenes*, with only 12% being susceptible to ≤2.0 µg/ml. By contrast, daptomycin was the most active agent against *C. jeikeium* with a geometric mean MIC of 0.25 µg/ml.

C. Effect of Ca^{++} levels on daptomycin disk diffusion tests:

In testing Ca^{++} levels of 6 different lots of Mueller-Hinton agar we found five lots to contain 26-36 µg/ml and one lot to contain 6 µg/ml. The QC strain *S. aureus* ATCC 25923 gave 6 mm smaller zones around the daptomycin 30 µg disk on the medium with 6 µg/ml of Ca^{++} than on the other five lots (Table 2). Figure 2 graphically displays the distribution of daptomycin zone sizes for 756 staphylococci and enterococci tested on the lot of agar with 6 µg/ml of Ca^{++} vs. the lot with 28 µg/ml. There was an average of 6 mm difference in zone sizes between the two lots of media.

D. Daptomycin disk test interpretive criteria:

Figure 3 is a scattergram of daptomycin MICs and disk diffusion zone diameters. Using provisional breakpoints, there were no major or very major discrepancies and 13 (1.8%) minor discrepancies. The low discrepancy rate is due to the absence of resistant organisms. Two of the minor discrepancies were enterococci with intermediate MICs and susceptible zone diameters.

E. Quality control results:

Table 3 shows the proposed QC ranges developed from the 10-laboratory QC study. The percentage of results falling within these ranges varied from 95.3 to 99.7. These proposed ranges have been approved by the NCCLS.

Conclusions

1. **Daptomycin *in vitro* activity is significantly affected by Ca^{++} concentration in the test medium, whether tested by broth microdilution or disk diffusion.**
2. **We recommend physiologic concentrations of Ca^{++} in broth for microdilution tests of daptomycin susceptibility.**
3. **Because of the variation in Ca^{++} content of different lots of Mueller-Hinton agar, QC testing will be essential for daptomycin susceptibility tests by disk diffusion.**
4. **For daptomycin MIC breakpoints of S ≤2.0 µg/ml and R ≥8.0 µg/ml, we recommend zone diameter breakpoints of S ≥16 mm and R ≤12 mm.**
5. **Daptomycin QC ranges for four QC strains have been determined.**
6. **Daptomycin was highly active against 513 Gram-positive bacteria tested, including MRSA and VRE.**
7. **Final interpretive criteria shall await clinical information, especially concerning the response of enterococcal infections.**

#1003

Daptomycin Efficacy Against Vancomycin-Resistant *Enterococcus faecalis* (VRE)–Induced Pyelonephritis in the Mouse

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Abstract

Daptomycin is a novel lipopeptide antibiotic derived from *Streptomyces roseosporus*, with potent bactericidal activity *in vitro* against gram-positive organisms, including MRSA and VRE. Daptomycin is primarily eliminated from the body via the urine, suggesting that it may be useful in the treatment of gram-positive urinary tract infections (UTI). This study examined the efficacy of daptomycin in a murine model of pyelonephritis. CF-1 female mice were pretreated with intravenous (IV) carrageenan at 6.4 mg/mouse for conditioning of the kidneys 7 days prior to IV inoculation with 3×10^8 cfu of a clinical isolate of VRE. Starting 1 h after the inoculation, the mice were treated with daptomycin at 1, 5, and 25 mg/kg subcutaneously (SC) or IV, twice a day for 3 days. Kidney homogenates of each mouse were prepared and VRE counts (cfu/pair kidneys) were determined as shown below:

Route of Administration	Daptomycin Dose (mg/kg)			
	1	5	25	
SC	3.1×10^8	7.1×10^6	3.0×10^5	5.0×10^3
IV	2.5×10^8	9.7×10^6	2.7×10^5	1.4×10^3

Daptomycin treatment was associated with a dose-dependent reduction in VRE counts in the infected kidneys, with approximately 70%, 90%, and >99.9% eradication at dose levels of 1, 5, and 25 mg/kg, respectively, independent of route. There was no change in daptomycin MIC for organisms recovered from kidney homogenates. These results demonstrate the efficacy of daptomycin against VRE-induced pyelonephritis in the murine model of UTI.

Introduction

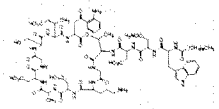
Daptomycin is a cyclic lipopeptide antibiotic derived from *Streptomyces roseosporus*. The drug acts on a unique cell wall/membrane target. It exhibits potent bactericidal activity *in vitro* against gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (Table 1). Daptomycin is primarily eliminated from the body via the urine, suggesting that it may be useful in the treatment of gram-positive urinary tract infections (UTI). The present study examined the therapeutic effect of daptomycin against a urinary tract infection experimentally induced by VRE, an emerging pathogen resistant to many existing antibiotics.

Study Objectives

1. **To examine the efficacy of daptomycin in a murine model of pyelonephritis induced by vancomycin-resistant *Enterococcus faecalis*.**
2. **To explore the pharmacokinetic profile of daptomycin in the mouse.**

Daptomycin

Chemical Structure:



Class of Drug: Lipopeptide, MW ~1620, water-soluble

Possible Mechanisms of Action:

- Inhibits bacterial lipoteichoic acid synthesis
- Disrupts membrane potential

Table 1. *In Vitro* Susceptibility to Daptomycin and Vancomycin of Isolates from 1997/1998*

Organism	No.	MIC_{90} (mg/L)		Range (mg/L)	
		Daptomycin	Vancomycin	Daptomycin	Vancomycin
S. aureus	27	1	1	0.06–1	0.5–2
MRSA	54	1	1	0.5–1	1–2
VISA**	12	1	4	<0.25–2	2–8
Staphylococcus spp. (coag.-neg.)	29	1	4	0.25–2	2–4
E. faecalis	20	1	2	0.25–2	0.5–2
E. faecium-VRE	22	1	512	0.5–2	>64–1024
S. pneumoniae PRSP	15 40	0.06 1	0.25 0.5	0.0075–0.06 0.06–1	0.015–0.5 0.06–1
S. pyogenes	10	0.125	0.5	0.075–0.5	0.25–1

*Data from NCMIC ® Syndromes. **Data from CDC (F. Tenover).

Experimental Procedures

A. Efficacy of daptomycin in murine urinary tract infection (UTI)

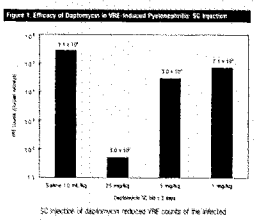
1. The study used CF-1 female mice (Charles River Lab, MA) weighing 22–25 g. Each dose group included 4 mice. Water and Agway rodent chow were provided *ad libitum* throughout the study.
2. On day 0, all mice were injected IV with 0.2 mL of 0.2% Carrageenan Lambda (Sigma Chemical Co., St. Louis, MO; C-3889) to damage kidney function and increase susceptibility to bacterial infection.
3. The pathogen used to induce UTI was a clinical isolate of vancomycin-resistant *E. faecalis* (Cubist #80 Daptomycin MIC = 2.5 µg/mL) obtained from New England Medical Center. On day 6, the bacterial strain was cultured in Brain-Heart Infusion broth (BBL, MD) at 37°C for 18 h, and following dilution in fresh medium, the inoculum inoculum was adjusted by measuring the optical density (OD at 600 nm.
4. UTI caused by vancomycin-resistant *E. faecalis* was introduced by a modification of the method of Menabrekk et al (Efficacy of A81-773, a 2-cyanobow antimicrobial, against enterococci, *Escherichia coli*, and *Pseudomonas aeruginosa* in experimental murine pyelonephritis, *J Antimicrob Chemother* 33:841–852, 1994). On day 0, all mice were injected IV with 0.2 mL of the culture (3×10^8 cfu/mouse). Through the tail vein. One hour after bacterial inoculation, group 1 mice were injected (IV or SC) with 10 mL/kg saline, and groups 2, 3, and 4 were injected with daptomycin (IV or SC) at 25, 5, and 1 mg/kg, respectively. The treatment was given twice a day for 3 consecutive days.
5. All mice were sacrificed on day 10. Their kidneys were removed aseptically and homogenized with 4 mL of Trypticase Soy broth (30 g/L, BBL). The homogenates were diluted in Brain-Heart Infusion medium and plated on Enterococcosel agar (BBL, Macrobiology Systems, MD) containing 5 µg/mL vancomycin. VRE counts were determined, and the bacterial titer of each pair of kidneys was calculated and averaged for each group. Counts <20 cfu per pair of kidneys were considered sterilized. Comparisons were made between control and daptomycin-treated groups.

B. Pharmacokinetic evaluation of daptomycin (IV and SC) in the mouse

1. Fifteen 7 or 3 CD-1 female mice, weighing 20–25 g each, were studied per time point.
2. Daptomycin (100 mg/kg) was administered by subcutaneous (SC) or intravenous (IV) injection. Daptomycin is highly soluble in saline.
   - Schedule for SC administration: At time 0, blood samples were collected from 3 naive mice. Another 24 mice were injected SC with 100 mg/kg daptomycin. Blood samples were collected through cardiac puncture from 3 mice at each of the following time points: 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h.
   - Schedule for IV administration: At time 0, blood samples were collected from 3 naive mice. Another 18 mice were injected IV with 100 mg/kg daptomycin. Blood samples were collected through cardiac puncture from 2 mice at each of the following time points: 2 min, 5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, and 6 h.
3. About 10.8–1 mL from each mouse was collected into a syringe containing 50 units of heparin and centrifuged immediately after sampling. Plasma samples were stored at −20°C before HPLC measurement of daptomycin.

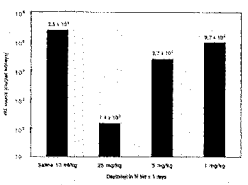
Results

Figure 1. Efficacy of Daptomycin in VRE-Induced Pyelonephritis: SC Injection



SC injection of daptomycin reduced VRE counts of the infected kidneys in a dose-dependent manner.

Figure 2. Efficacy of Daptomycin in VRE-Induced Pyelonephritis: IV Injection



IV injection of daptomycin reduced VRE counts of the infected kidneys in a dose-dependent manner.

Table 2. Summary of Efficacy Studies of Daptomycin Against VRE-Induced Pyelonephritis

Route of Administration	Daptomycin Dose (mg/kg)			
	1	5	25	
SC	3.1×10^8	7.1×10^6	3.0×10^5	5.0×10^3
IV	2.5×10^8	9.7×10^6	2.7×10^5	1.4×10^3

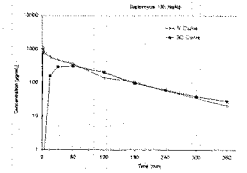
This table shows the VRE counts of the kidney homogenates.

Table 3. Summary of Pharmacokinetic Studies of Daptomycin in CD-1 Mice

Dose	Intravenous 100 mg/kg, single dose	Subcutaneous 100 mg/kg, single
C_{max} (µg/mL)	1402	318
T_{max} (min)	N/A	60
Elimination Half-life (min)	75	52
AUC (µg*min/mL)	83074	48172
Bioavailability (%)	N/A	78.4
$Clearance_{Body}$ (mL/min/kg)	1.6	2.1
Vol. of Distribution (mL/kg)	162	250

N/A: not applicable

Figure 3. Comparison of the Pharmacokinetics of IV and SC Injection of Daptomycin: CD-1 Mice



Summary and Conclusions

1. **Daptomycin treatment caused a dose-dependent reduction in VRE in the infected kidneys of mice.**
2. **These results demonstrate the efficacy of daptomycin against VRE-induced pyelonephritis in the murine model of UTI.**
3. **Daptomycin pharmacokinetic profile following SC injection is consistent with IV profile; SC bioavailability was high (78%).**

#1770

The Pharmacodynamics of Daptomycin as Determined for *Staphylococcus aureus* in a Mouse Thigh Infection Model

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Abstract

Daptomycin is a lipopeptide antibiotic with activity against Gram-positive bacteria, including *Staphylococcus aureus*. We defined the pharmacodynamic parameters of daptomycin for *S. aureus* using in vitro methods and a neutropenic mouse thigh infection model developed by Craig et al.[1] In Mueller-Hinton broth, the MIC and MBC for the *S. aureus* isolate employed were 0.2 and 1.0 µg/ml, respectively. By an ultracentrifugation method, the protein binding of daptomycin was 88 to 92% in mouse serum. Single-dose pharmacokinetic studies in infected mice showed a linear relationship between dose vs. Cmax and dose vs. AUC for drug given IP. The serum half-life of daptomycin in infected mice was approx. 1.8 h. In single-dose, dose-ranging studies, daptomycin demonstrated a dose response of effect described by an Emax curve. Control growth was 8.29 log_{10} CFU/mL Emax was 3.97 log_{10} CFU/mL. The ED_{50} was 3.7 mg/kg IP (SD 1.21 mg/kg). The model fit the data well (r^2 = 0.972, P << 0.001). Dose-fractionation studies of schedules of q8h, q12h, and q24h were performed at doses of 2, 4, 8, and 16 mg/kg/day. No difference in effect was seen at any dose level by schedule, indicating that AUC/MIC ratio is the dynamically linked variable. The static dose was 7.1 mg/kg. The 90% Bayes dose was 23.7 mg/kg. As protein binding is 90% in man, no transformations need be applied to the data to scale the results to man. These mg/kg/day doses correspond to AUCs of 58.6 and 114.8 µg/ml•h. As the median daptomycin plasma clearance in man is 8.63 L/h, 4 x or 8 mg/kg dose once daily will considerably exceed the target AUC, even for the 90% Bayes effect. Daptomycin should be vigorously evaluated in clinical trials in patients with *S. aureus* infections.

Introduction

The incidence of community- and nosocomially-acquired infections due to the bacterium *Staphylococcus aureus* is rising.[2] From 1990 to 1992 this microorganism was the most common cause of nosocomial pneumonias and surgical wound infections.[3] Although the rate staphylococcal beta lactam antibiotics are the most active agents available for the treatment of methicillin-susceptible *S. aureus*, the National Nosocomial Infections Surveillance System of the CDC reports that in 1997 56.2% of *S. aureus* isolates were resistant to these agents.[4] Vancomycin is considered the "drug of choice" for the treatment of methicillin-resistant *S. aureus* (MRSA) infections. However, this drug is slowly bactericidal and is associated with higher failure and relapse rates than beta-lactam therapy for the treatment of *S. aureus* endocarditis. Also, strains of *S. aureus* demonstrating decreased susceptibility to vancomycin have been described in Japan[5] and the United States.[6]

Daptomycin is an acidic lipopeptide antibiotic that is active against Gram-positive bacteria, including *S. aureus* and enterococci, including isolates with decreased susceptibilities to vancomycin. In previous studies, daptomycin compared favorably with vancomycin in the treatment of soft tissue and skin infections due to Gram-positive bacteria. However, in one clinical study daptomycin was found to be less active than vancomycin for the treatment of *S. aureus* endocarditis. Importantly, that clinical study did not fully correlate the dose-range activity and the pharmacodynamics of daptomycin in choosing the study dose. Furthermore, the impact of the high protein binding of daptomycin on the drug's in vivo activity was not considered. Thus, the efficacy of daptomycin in the treatment of clinical infections due to Gram-positive bacteria was not fully evaluated.

In the current study, we used a murine neutropenic thigh model[1] of *S. aureus* infection to define the pharmacodynamics and complete dose-range activity of daptomycin in infected mice. Using doses and dosing schedules that mimicked the duration of time that dosing-cycle remains above the MIC for the infecting pathogen (Time > MIC), the AUC/MIC ratio, or the Cmax/MIC ratio, we determined which pharmacodynamic parameter predicted best outcome.

Materials and Methods

Antimicrobial agent. Daptomycin powder (lot 442P01D1-35-1, purity 97.1%) was supplied by Cubist Pharmaceuticals, Inc. (Cambridge, MA). The drug was prepared in Mueller Hinton broth (Difco, Detroit, MI) supplemented with 50 mg/l of calcium (Ca HMB) in mouse serum (Sterile Biologicals, Inc., Oskemos, MO). The concentration of ionized free calcium in reduced zero was quantified and was found to be within the range normally measured in human sera.

Microorganism. *S. aureus* ATCC 29213 was used throughout the studies. Fresh isolates were grown on blood agar plates for 24 h at 35°C before each study.

Susceptibility studies. MIC and MBC studies were conducted for *S. aureus* ATCC 29213 in Ca HMB and 100% mouse serum using a macrobroth dilution method described by NCCLS. The concentration of bacteria in each macrobroth dilution tube was 5 x 10^5 CFU/ml of Ca HMB or mouse serum.

Protein binding studies. The extent to which daptomycin binds to mouse serum proteins was determined using an ultracentrifugation method described by Craig and Suh.[7] Briefly, daptomycin was added to 100% mouse serum to final concentrations between 3 and 80 µg/ml. An aliquot was taken from each sample and the remainder of the samples were ultracentrifuged at 295,000 x g at 25°C for 1 h. The supernatant was collected. The concentrations of daptomycin in pre-centrifuged serum samples and supernatants were measured using a bioassay as assay were defined. The percent of daptomycin bound to serum proteins was calculated using the formula:

$$\text{Percent bound} = [\text{serum drug concentration} - \text{drug concentration in supernatant}] / \text{serum drug concentration}$$

Daptomycin bioassays. The concentrations of daptomycin in Ca HMB and mouse serum were measured using a bioassay assay. Micrococcus luteus ATCC 7468 was used as the assay organism. Four inches containing 12 inches of organism were prepared in beef infusion agar (Difco, Detroit, MI) supplemented with 2% NaCl, 0.5% $CaCl_2$, and 0.1% uric acid. Twenty microliter amounts of sample or standards were pipetted onto the wells. After the plates were incubated overnight the diameters of inhibition for samples and standards were measured. Daptomycin concentrations in samples were calculated by using the data from the curves derived from the drug standards. The standard curve was linear between 0.2 and 500 µg/ml when the standards were prepared in saline or Ca HMB (r^2 > 0.99 with a daily CV < 6.7%, inter-day CV < 8.4%). In serum the standard curve was linear between 0.4 and 200 µg/ml (r^2 > 0.99, intra-day CV < 5.8%, inter-day CV < 8.4%).

Animals. Female, 24–26 g ICR/Swiss mice were used (Charles River, Wilmington, MA). They received food and water ad libitum.

Neutropenic murine *S. aureus* thigh infection model. We adapted the mouse thigh infection model described by Craig[1] to examine the relationship between daptomycin exposure and reduction in the density of *S. aureus* in the thigh muscle of neutropenic mice. Mice were given cyclophosphamide 150 mg/kg IP 3 days and 100 mg/kg of cyclophosphamide IP 2 h prior to bacterial inoculation. Thirty-four hours after antibiotic therapy was begun, the mice were sacrificed. Eight additional infected mice were sacrificed just before the other groups received daptomycin to define the bacterial density in thigh muscles at the start of therapy. Both posterior thigh muscles[8] were aseptically collected from sacrificed mice. The tissue was weighed, homogenized, and serially diluted. Aliquots from each tube of the dilution series were plated onto beef infusion agar (Hill, Detroit, MI) at 48 h of incubation. The colonies were read. The density of bacteria in each posterior thigh muscle was calculated. Drug carryover was not seen for the doses of drug used.

Dose-ranging validation study. A second single-dose dose-ranging study was conducted to validate the results of the first dose-ranging study. The doses of daptomycin used corresponded to the ED_{50}, ED_{75}, ED_{90}, and ED_{99} doses derived from the first trial. Also, 2, 0.1, and 40 mg/kg doses were randomized to fully delineate the sigmoid Emax dose response relationship. Otherwise, the experimental methods used were as described for the first dose-response trial. There were 5 animals per group.

Single-dose pharmacokinetics of daptomycin in infected mice. Two hours after mice were infected, the animals were injected IP with 2 to 20 mg/kg of daptomycin. Three animals from each group were sacrificed 0.25 h to 8 h later for blood collection. The concentrations of drug in serum samples were determined using the microbiological bioassay described above.

Effect of dose-scheduling of daptomycin on bacterial densities in infected mice. Simultaneously with the dose-range validation study, we conducted dose-fractionation studies to determine if the pharmacodynamic parameter that best predicts the maximal benefit of daptomycin was the AUC/MIC ratio, Cmax/MIC ratio, or the Time > MIC. The doses selected for the dose-fractionation study were those that, based on the first dose-range study, were predicted to fall on the steep portion of the sigmoid Emax dose response curve (eg, the ED_{50}, ED_{75}, and ED_{90} doses). Each total dose of daptomycin selected was given IP to groups of infected mice as either a single injection, two equally divided doses given 12 h apart, or four equally divided doses given 6 h apart. An additional group of infected mice received saline and served as controls. There were 5 mice per group.

The first dose of daptomycin was administered to animals 2 h after the mice were inoculated in both posterior thigh muscles with *S. aureus*. Twenty four hours after the first dose of daptomycin or saline was given, the animals were sacrificed. Both posterior thigh muscles were collected from each mouse for quantitative cultures. The results for groups that received the same total dose of daptomycin were compared with each other and with the control group.

Predictive activity of daptomycin in man. The dose-response, pharmacokinetic, and pharmacodynamic data generated in this project were combined with pharmacokinetic data derived from human volunteers (data provided by BLD by Cubist Pharmaceuticals) given various dosages of daptomycin IV. The data were transformed to predict the likelihood of achieving stasis and ED_{50} effects in humans who are given these doses of daptomycin IV for the treatment of systemic *S. aureus* infection. The human pharmacokinetic data consist of 1521 serum samples collected over time from 80 normal volunteers and individuals with renal failure. Normal volunteers received multiple doses of drug prior to collection of serum samples; subjects with renal failure received one dose. The mean drug clearance and area under the concentration time curve (AUC) associated with each dose were calculated from the human data. With consideration of the degree to which daptomycin binds to mouse and human serum proteins, the mouse data were transformed to predict the likelihood of achieving stasis and an 90% of 3 log reduction in *S. aureus* densities in infections in humans. The model predictions were based on 1000 pharmacokinetic simulations conducted on the human data (done in triplicate).

Pharmacokinetic analysis. Pharmacokinetic analysis of the serum sample daptomycin concentration time relationships was performed with a nonlinear least squares regression program, NONMEM (Metrumath Scientific Software, Salt Lake City, UT). To determine the AUC the trapezoidal method was used from time zero to the last time point, then extrapolated to infinity.

Statistical analysis. The relationship between the dosage of daptomycin administered and the bacterial densities in thigh muscles of infected mice was evaluated by an analysis of sigmoid Emax dose response model using the identification module of the ADAPT II package of programs of D'Argenio and Schumitzky Biomedical Simulations Resource, University of Southern California, Los Angeles, CA. Weighting was by the inverse of the observation variance. The significance of differences between two group densities between groups that received the same dose dose of daptomycin at one time, a *t* test (Instat) Jones was evaluated by ANOVA. A difference was considered statistically significant at $P < 0.05$.

Results

Table 1. Protein binding studies in 100% mouse serum

Concentration (µg/ml) of Daptomycin in Serum	Percent Bound to Serum Proteins (± 1 SD)
2	91.8 ± 1.3
4	90.3 ± 2.5
8	91.3 ± 0.6
20	91.3 ± 0.6
40	92.5 ± 1.7
60	91.7 ± 1.5
80	91.2 ± 1.3

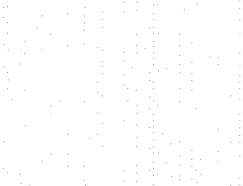
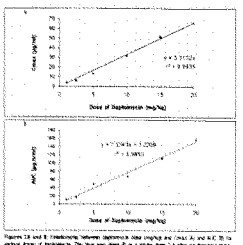


Figure 1. Single-dose concentration vs. time pharmacokinetic profiles for incremental doses (mg/kg) of daptomycin in infected mice for drug given IP.



Figures 2A and B. Relationship between daptomycin dose (mg/kg) and Cmax (A) and AUC (B) for various doses of daptomycin. The drug was given IP to a single dose 2 h after neutropenic mice were inoculated IM with *S. aureus*.

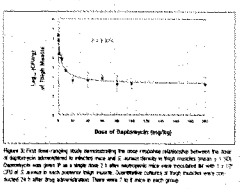


Figure 3. Dose-ranging study demonstrating the dose-response relationship between the dose of daptomycin administered to infected mice and *S. aureus* density in thigh muscles (mean ± 1 SD). Daptomycin was given IP as a single dose 2 h after neutropenic mice were inoculated IM with 1 x 10^7 CFU of *S. aureus* in each posterior thigh muscle. Quantitative cultures of thigh muscles were conducted 24 h after drug administration. There were 7 to 8 mice in each group.

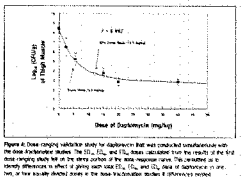


Figure 4. Dose-ranging validation study for daptomycin that was conducted simultaneously with the dose-fractionation studies. The ED_{50}, ED_{75}, and ED_{90} doses calculated from the results of the first dose-ranging study fell on the steep portion of the dose-response curve. The animals ad libitum differences in effect of giving each total ED_{50}, ED_{75}, and ED_{90} dose of daptomycin in one, two, or four equally divided doses in the dose-fractionation studies if differences existed.

Table 2. Calculated pharmacodynamic variables for three total dosages of daptomycin administered in one, two, or four equally divided doses

Total Dose of Daptomycin in mg/kg/24 h	Regimen	Cmax/MIC Ratio	AUC/MIC Ratio	Time > MIC/24 h
2.5	2.5 mg/kg (1 dose)	5.29	30.36	5.2
	1.25 mg/kg q12h (2 doses)	4.14	31.18	9.1
	0.625 mg/kg q6h (4 doses)	2.67	29.72	17.2
5.0	5.0 mg/kg (1 dose)	10.54	62.61	6.08
	2.5 mg/kg q12h (2 doses)	8.07	64.83	10.17
	1.0 mg/kg q6h (4 doses)	4.62	67.08	18.34
10.0	10.0 mg/kg (1 dose)	20.65	114.74	9.44
	5.0 mg/kg q12h (2 doses)	14.62	115.48	12.12
	2.25 mg/kg q6h (4 doses)	12.43	117.30	21.34

a. The first dose was administered 2 h after infection.
b. The MIC for *S. aureus* ATCC 29213 was 1.0 µg/ml in 100% mouse serum by NCCLS macrobroth dilution method.

Table 3. *S. aureus* densities (log_{10} CFU/g ± 1 SD) in thigh muscles of mice that were treated with various total doses of daptomycin, administered in one, two, or four divided doses

Total Daptomycin Dosage (mg/kg)	Schedule of Administration: 1 dose	2 divided doses*	4 divided doses*	P Value†
2.5	5.34 ± 0.98	5.83 ± 0.58	6.61 ± 0.43	0.04
5.0	5.12 ± 2.06	4.96 ± 2.59	5.42 ± 0.52	0.72
10.0	2.21 ± 3.31	1.82 ± 2.55	5.66 ± 0.43	0.08

a. By ANOVA, $P < 0.05$ was considered statistically significant.
b. These doses were given 2 h apart.
c. These doses were given 6 h apart.

Results and Discussion

MIC and MBC of daptomycin for *S. aureus* in defined media and mouse serum. For *S. aureus* ATCC 29213, the median MIC and MBC studies in Ca HMB were 1.2 and 1.0 µg/ml, respectively. In 100% mouse serum, the MIC was 1.0 µg/ml (range: 0.8 to 1.0 µg/ml).

Protein binding studies. Table 1 shows that 90.3 to 92.5% of daptomycin was bound to mouse serum proteins. The degree of protein binding in comparison with the 90 to 94% rate described for human plasma.[9]

Single-dose pharmacokinetics of daptomycin in infected mice. As shown in Figure 1, the Cmax was seen 30 minutes after daptomycin was administered. Both the Cmax and AUC increased in proportion to the dose of daptomycin administered. Figures 2A and B, respectively. The pharmacokinetics of daptomycin were best described by a two-compartment model with a terminal half-life of approximately 1.8 h.

Single-dose, dose-response study in a neutropenic murine thigh model of *S. aureus* infections. In Figure 3, the dose-response relationship for daptomycin is best described by an Emax curve (r^2 = 0.972, P << 0.001) with the calculated maximal effect seen with > 40 mg/kg of daptomycin. At 24 h, an Emax dose resulted in a 4.3 log_{10} decrease in bacterial density vs. no treatment. Quantitative cultures demonstrated that the mean bacterial density in the posterior thigh muscles was 1.1 x 10^9 CFU/g at the time of *S. aureus* inoculation. The "static dose," which is believed at the dose of drug that results in the same bacterial concentration in the infected site at the 24 h endpoint and at 0 h, was 7.1 mg/kg (Figure 3). The ED_{50}, ED_{75}, ED_{90}, and ED_{99} were 3.7, 5.7, 7.5, 10.0, and 12.7 mg/kg of daptomycin, respectively.

Exclusive of animals that received doses of daptomycin at 1.5 mg/kg IP (redose) and acted well throughout the study (data not shown), there was no clinical evidence of drug toxicity for the doses examined.

Dose-ranging validation study. The results shown in Figure 4 were similar to those of the first study in the validation study. Thigh muscles in the control group had a mean of 8.21 log_{10} CFU of *S. aureus* per gram of tissue. The Emax doses were associated with *S. aureus* densities of 1.76 log_{10} CFU/g. Thus, Cubist doses resulted in a 6.76 log_{10} CFU/g reduction in density over 24 h. The predicted ED_{50}, ED_{75}, ED_{90}, and ED_{99} derived from the first dose-response study fell on the steep portion of the dose-response curve of the validation study.

Pharmacodynamic variables for the three daptomycin regimens. Area the linear regressions for dose vs. Cmax and dose vs. AUC (Figures 2A and B), and pharmacokinetic data for dose vs. Time > MIC, we derived the relationships between these pharmacokinetic parameters and drug exposures for 2.5, 5.0, and 10.0 mg/kg of daptomycin (Table 2). When examining the Cmax/MIC ratio, it is clear that once-daily administration of a total dose of daptomycin produced the maximal index. For Time > MIC, the most fractionated schedule (ie, the q6h regimen) produced the longest Time > MIC, while once-daily dosing produced the shortest time. As daptomycin has linear pharmacokinetics, one would expect that the AUC for a full 24 h period would be similar no matter the schedule of administration.

Dose-fractionation trials. Post hoc analysis of the results of the dose-ranging validation study conducted simultaneously demonstrated that all the doses of daptomycin chosen for this dose-fractionation study lay on the steep portion of the sigmoid Emax dose response curve (Figure 4). In the validation study the "static dose" of 2.5, 5.0, and 10.0 mg/kg of daptomycin corresponded to the ED_{50}, ED_{75}, and ED_{90}, respectively, as demonstrated in Table 3. The bacterial densities were similar for groups that received the same total dose of daptomycin in one, two, or four equally divided doses over 24 h in the dose fractionation study. These results clearly demonstrate that, for daptomycin, the AUC/MIC ratio is the pharmacodynamically linked variable.

Probability of achieving the stasis and 90% of the Emax effect in humans treated with 4 or 8 mg/kg of daptomycin IV. Based on previously pharmacokinetic data generated in humans conducted by Cubist Pharmaceuticals, the median and mean clearance of drug in man are 8.63061 and 0.460 L/h. For a 70 kg man, the 4 and 8 mg/kg doses would result in 24 h AUCs of 444.1 and 888.3 µg/ml, respectively.

Our data from mice show that a static dose of 7.1 mg/kg results in an AUC of 58.6 µg/ml. The ED_{90} dose of 10.7 mg/kg is associated with an AUC of 114.8 µg/ml. The problem is that daptomycin is human and mouse serum proteins is similar. Therefore, no translation need be applied to the data to scale it to man.

A mathematical model, using 1000 Monte Carlo simulations in each of three dosing periods, that for our *S. aureus* isolate MIC = 0.2 µg/ml, in Ca HMB, the 4 and 8 mg/kg doses will exceed the AUC associated with the ED_{50} in 76.7% of 3000 simulations.

Because the MIC of daptomycin for *S. aureus* is 1 µg/ml (provided by Cubist Pharmaceuticals) we predict that 8 mg/kg dose will achieve or exceed the stasis AUC and 90% Emax AUC for 63.4% and 50.0% of 3000 simulations, respectively.

Conclusions

The MIC and MBC of daptomycin were 0.2 and 1.0 µg/ml in cation-supplemented Mueller-Hinton broth. In 100% mouse serum, the MIC was 1.0 µg/ml.

Daptomycin is highly bound to mouse serum (range: 90.3 to 92.5%). The change is similar to that described for human serum.

Single-dose, dose-ranging studies demonstrate that the activity of daptomycin is best described by a sigmoid Emax dose-response curve. At 24 h, doses associated with the Emax effect resulted in a 4.3 log_{10} decrease in bacterial densities in the thigh muscles vs. no treatment. There was no clinical evidence of toxicity with doses of daptomycin as high as 250 mg/kg IP.

The ED_{50}, ED_{75}, ED_{90}, and ED_{99} are 3.5, 5.4, 15.8, and 33.7 mg/kg, respectively.

Pharmacokinetic studies demonstrate that a linear relationship exists between dose and AUC or Cmax for the range of doses examined. The serum half-life of daptomycin in infected mice is approximately 1.8 h.

Dose-fractionation studies demonstrate that the pharmacodynamic parameter that predicts outcome for daptomycin is the AUC/MIC ratio.

For the *S. aureus* strain evaluated (MIC of 0.2 µg/ml in defined medium), the bacteriostatic and pharmacodynamic data suggest that 4 and 8 mg/kg of daptomycin will achieve stasis AUCs in humans that will exceed the AUC associated with 90% of Emax in 99.7% of 3000 simulations. For an *S. aureus* MIC, of 1 µg/ml, we predict that 8 mg/kg dose will achieve or exceed the stasis and 90% Emax AUC for 63.4% and 50.0% of 3000 simulations, respectively.

Daptomycin should be a useful drug in the treatment of *S. aureus* infections in man.

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#1769

In-Vivo Pharmacodynamic Activity of Daptomycin (DAP) Against Multiple Bacterial Pathogens

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Abstract

Background: The peak/MIC and 24-hr AUC/MIC are the PK/PD parameters that best correlate with in-vivo activity of DAP. We used the neutropenic murine thigh-infection model to determine if the magnitude of the peak/MIC and 24-hr AUC/MIC needed for efficacy of DAP varied among pathogens (including resistant strains).

Methods: Mice had $10^{6.4-7.8}$ cfu/thigh of 4 isolates of *S. aureus* (1 MRSA) and 9 isolates of *S. pneumoniae* (2 PISP, 8 PRSP) when treated for 24 hrs with 0.38–400 mg/kg of DAP every 12 hrs. Serum levels were determined by microbiologic assay. A sigmoid dose-response model was used to estimate the dose (mg/kg/24 hrs) required to achieve a net bacteriostatic effect over 24 hrs.

Results: PK studies exhibited peak/dose values of 2.8–5.2, AUC/dose values of 9.2–9.5, and half-lives of 1.1–1.2 hrs. Protein binding was 90%. MICs ranged from 0.12–0.5 mg/L. Static doses for the various organisms ranged from 1.0–28 mg/kg/day. Mean peak (free)/MIC and 24-hr AUC (free)/MIC values were 7.1 and 44 for *S. aureus* and 2.4 and 16 for *S. pneumoniae*. The differences were significant. Methicillin and penicillin resistance did not alter the magnitude of peak/MIC and AUC/MIC required for efficacy.

Conclusions: The peak/MIC and 24-hr AUC/MIC of DAP required for in-vivo efficacy were relatively similar among various pathogens and were not altered by drug resistance.

Background

Previous studies with daptomycin demonstrated that the peak concentration and the AUC were the parameters that best correlated with in-vivo efficacy against strains of MSSA and MRSA in the neutropenic murine thigh-infection model (Abstract 154, ICAAC 1987). We used the same model to determine the magnitude of the peak/MIC and the 24-hr AUC/MIC ratios required for efficacy of daptomycin against multiple strains of *S. pneumoniae* and *S. aureus*, including isolates resistant to penicillin and methicillin.

Materials and Methods

Bacteria: The study organisms consisted of nine strains of *Streptococcus pneumoniae* (2 strains of PISP and 2 strains of PRSP) and four strains of *Staphylococcus aureus* (1 strain of MRSA).

Mice: SPF female ICR/Swiss mice weighing 23–25 g.

Infection Models: Mice received two injections of cyclophosphamide (150 mg/kg 4 days before study and 100 mg/kg 1 day before study). Thigh muscle was infected by direct injection of 0.1 ml of a 1:10 dilution of 10^8 bacteria. Animals had $10^{6.4-7.8}$ cfu/thigh at the start of therapy.

Antimicrobial treatment was started 2 hrs after thigh infection and continued for 24 hrs. Doses given sq ranged from 0.38–400 mg/kg every 12 hrs. Two mice were used for each dosing regimen.

Thigh muscles and lungs were removed and homogenized in iced saline. Aliquots of four serial 10-fold dilutions were plated on blood or MH agar for cfu determinations.

Pharmacokinetics: Serum samples were obtained at various time points following single sc doses of 10 and 40 mg/kg of daptomycin. Serum concentrations were determined by microbiologic assay. Protein binding was determined by ultrafiltration.

Data Analysis: An Emax dose-response model based on the Hill equation was used to calculate the doses required to produce a static effect and 1 and 2 log cfu reductions below the starting inoculum.

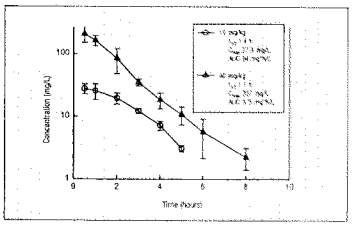
$E=E_{max}*D^n/(D^n+ED_{50}^n)$, where E=effect, E_{max}=maximum effect, D=dose, ED_{50}=dose that achieves 50% of the maximum effect, and n=slope of the dose-effect curve.

Results

The MICs for daptomycin ranged from 0.12 to 0.5 mg/L (see Table 1).

The time course of serum concentrations is shown in Figure 1. PK analysis revealed peak/dose values of 2.8–5.2, AUC/dose values of 9.2–9.5, and half-lives of 1.1 to 1.2 hrs. The protein binding of daptomycin in mouse serum was 90%.

Figure 1. Daptomycin Pharmacokinetics



The dose-response curves for 12-hourly administration of daptomycin with multiple strains of *S. pneumoniae* and *S. aureus* are shown in Figures 2 and 3, respectively. The dose-response curves for the various strains of *S. pneumoniae* were relatively similar. The dose-response curves for the four strains of *S. aureus* were almost identical.

Figure 2. Dose-Response Relationships for Daptomycin Against Strains of *S. aureus*

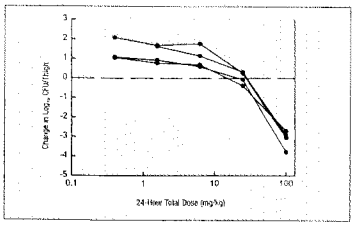
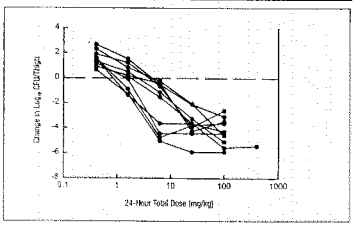


Figure 3. Dose-Response Relationships for Daptomycin Against Multiple Strains of *S. pneumoniae*



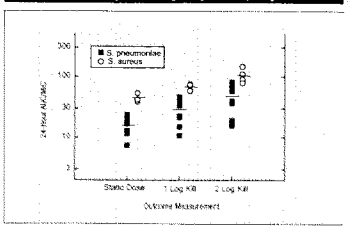
The magnitude of the 24-hr AUC/MIC and peak/MIC ratios associated with the doses required to produce a static effect or reduce cfu by 1 and 2 logs over 24 hours are listed in Table 1 and shown graphically in Figure 4. Although the static doses varied 28-fold and ranged from 1.0–28 mg/kg/day, the 24-hr AUC/MIC and peak/MIC values for these doses varied 7.1- to 7.9-fold, respectively.

The mean 24-hr AUC/MIC and peak/MIC values for *S. pneumoniae* (160 and 24 for total drug and 16 and 2.4 for free drug) were significantly lower ($p<0.05$) than those for *S. aureus* (438 and 71 for total drug and 44 and 7.1 for free drug). Thus, free drug concentrations need to average about 1 times the MIC over 24 hrs for *S. pneumoniae* and 2 times the MIC over 24 hrs for *S. aureus*. Penicillin and methicillin resistance did not alter the magnitude of the 24-hr AUC/MIC and peak/MIC ratios of daptomycin that were required for efficacy.

Table 1. Activity of Daptomycin (Total Drug) Against Multiple Organisms

Organism	MIC (mg/L)	24-H AUC/MIC Ratio			Peak/MIC Ratio		
		Static Dose	1 Log Kill	2 Log Kill	Static Dose	1 Log Kill	2 Log Kill
S. pneumoniae ATCC 10813	0.12	188	290	582	25.1	48.2	86.5
S. pneumoniae CDC 143	0.12	74.7	108	157	11.8	16.0	23.4
S. pneumoniae CDC 1283	0.12	203	346	594	30.5	51.5	88.1
S. pneumoniae CDC 1199	0.12	117	150	190	17.4	22.3	28.3
S. pneumoniae CDC 1396	0.12	237	467	815	35.5	69.5	121
S. pneumoniae CDC 673	0.25	159	373	673	29.8	55.5	100
S. pneumoniae CDC 1325	0.25	182	397	703	27.2	59.0	104
S. pneumoniae ATCC 49619	0.25	126	215	395	18.8	32.0	58.5
S. pneumoniae CDC 1020	0.25	129	224	369	19.4	33.5	55.4
Mean ± SD		**160 ± 51**	**290 ± 121**	**498 ± 131**	**24.0 ± 7.6**	**42.1 ± 17.2**	**73.9 ± 34.2**
95% CI		(72–316)	(100–660)	(117–813)	(11–44)	(15–98)	(20–204)
S. aureus ATCC 25923	0.5	268	384	898	59.0	109	197
S. aureus ATCC 33591	0.5	537	733	1349	93.9	147	264
S. aureus ATCC 29213	0.5	420	588	788	66.2	107	163
S. aureus ATCC 653Mo	0.5	459	750	1492	63.0	152	298
Mean ± SD		**438 ± 87**	**866 ± 87**	**1061 ± 296**	**70.6 ± 15.8**	**129 ± 24.1**	**255 ±104**
95% CI		(316–560)	(501–832)	(603–1738)	(47–102)	(86–184)	(114–507)

Figure 4. Relationship Between Daptomycin (Free Drug) 24-H AUC/MIC and 24-H Static Dose, 1 Log Kill and 2 Log Kill Against Multiple Organisms



Conclusions

The magnitude of the 24-hr AUC/MIC and peak/MIC of daptomycin that is required for efficacy varied 7- to 8-fold with multiple pathogens and was not altered by penicillin or methicillin resistance.

The AUC/MIC and peak/MIC ratios required for efficacy were about two-fold lower for *S. pneumoniae* than for *S. aureus*.

Free drug concentrations of daptomycin need to average from 1 to 2 times the MIC over 24 hrs to produce a bacteriostatic effect and 2 to 4 times the MIC over 24 hrs to produce over 99% killing.

Safdar et al, ICAAC 1999, Poster #1769

P0957A

Once-Daily Dosing Decreases Toxicity of Daptomycin

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Abstract

Daptomycin is a novel lipopeptide antibiotic with potent bactericidal activity against Gram-positive bacteria, including resistant strains. Skeletal muscle has been identified as the target organ of daptomycin toxicity. To investigate the potential effects of dose fractionation on toxicity, two studies were conducted in dogs comparing the effects of repeated intravenous administration once daily (q24h) versus every 8 hours (q8h). Muscle toxicity did not appear to be directly related to C_{max} since both serum creatine phosphokinase (CPK) increases and the incidence of myopathy observed at 25 mg/kg q8h were greater than at 75 mg/kg q24h despite the lower C_{max}. A 3- to 4-fold increase in peak CPK levels was observed when the dose interval was varied from q24h to q8h at a dose of either 5 mg/kg or 25 mg/kg. In contrast, no difference in CPK elevations was apparent when the dosing interval was held constant at 24 hours and the dose was varied from 25 to 75 mg/kg. Further, 5 mg/kg administered q24h caused no change in CPK, whereas a dose regimen of q8h was associated with a 3-fold increase in CPK at a comparable C_{max}. Similarly, toxicity was not related primarily to AUC_{24h}, since the toxicity observed at 25 mg/kg q8h was greater than at 75 mg/kg q24h, at approximately the same AUC. The data suggest that dosing interval has a greater influence on toxicity than did dose itself. Once daily administration is hypothesized to allow greater time between doses for non-toxic blood levels for repair of subclinical muscle damage associated with daptomycin. Further, studies in animal efficacy models have demonstrated that effectiveness of daptomycin is optimized by once-daily dosing since C_{max} was found to be the key PK parameter associated with eradication of infection (J Leggett et al., ICAAC abstract 1987). These results suggest that once-daily dosing can minimize daptomycin muscle toxicity, while optimizing its antimicrobial efficacy.

Objective and Hypotheses

Skeletal muscle toxicity was dose-limiting in clinical trials of daptomycin. Reversible effects of increased serum CPK and muscle weakness were observed after 8 days at a dose of 4 mg/kg administered every 12 hours (4 mg/kg q12h). A dose regimen of 3 mg/kg q12h was not associated with skeletal muscle effects; however, this dose regimen may be insufficient for efficacy against certain clinical indications. Therefore, in an effort to increase the safety margin and maximum total daily dose, two nonclinical studies were undertaken to determine whether the dosing interval affects toxicity. These studies were conducted in the dog since this species is the most predictive of clinical effects.

Objective:

To assess the relationship between pharmacokinetics (C_{max} and AUC_{24h}) and skeletal muscle toxicity, in order to determine the optimal clinical dosing regimen to minimize potential for skeletal muscle toxicity.

Study A Hypothesis:

Daptomycin-related skeletal muscle toxicity is related to C_{max} and not AUC. Therefore, fractionation of the daily dose into multiple administrations per day will reduce the toxicity.

Study B Hypothesis:

A threshold plasma concentration exists for daptomycin related skeletal muscle toxicity. Therefore, administration of the q24h no effect dose level (NOEL q24h) multiple times per day, such that plasma levels remain below the threshold, will not be associated with skeletal muscle toxicity.

Study Designs

- Dose regimens:

  Study A: saline q8h, 25 mg/kg q24h, and 75 mg/kg q24h (associated with skeletal muscle toxicity in previously conducted studies), and 25 mg/kg q8h

  Study B: saline q8h, 5 mg/kg q24h (approximate NOEL q24h), and 5 mg/kg q8h

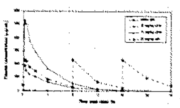
  Note: The q8h interval represents ~ 3 half-lives in dogs ($t_{1/2}$ ≈ 2.5 hours), and should have minimal impact on steady state C_{max} as compared to a q24h regimen.
- Duration of treatment: 20 days
- Dose groups: 4 male dogs/group

Results

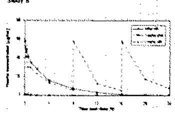
Plasma Levels

Steady state plasma concentrations of daptomycin on Day 18 of dosing were determined by HPLC.

Study A



Study B



C_{max} comparisons:

- Approximately same (1.23-fold higher) at 25 mg/kg q8h vs 25 mg/kg q24h
- Approximately 2.8-fold higher at 75 mg/kg q24h vs 25 mg/kg q8h or 25 mg/kg q24h

AUC comparisons:

- Approximately same (1.37-fold higher) at 25 mg/kg q8h vs 75 mg/kg q24h

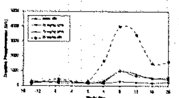
Pharmacokinetic comparisons:

- C_{max} same at two dose regimens
- AUC approximately 3-fold higher (2.3-fold) at 5 mg/kg q8h vs 5 mg/kg q24h

Serum Creatine Phosphokinase Levels

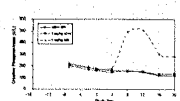
Serum CPK levels were determined at 2 hours post-dose as an indication of muscle toxicity.

Study A



- Throughout the treatment period, CPK levels at 25 mg/kg q8h ~ 4-fold higher than at either q24h regimen
- For all dose regimens, CPK peaked after ~ 1 week of treatment, then declined despite continued treatment

Study B



- No change in CPK levels at 5 mg/kg q24h as compared to saline control
- Throughout the treatment period, CPK levels at 5 mg/kg q8h elevated compared to 5 mg/kg q24h
- At 5 mg/kg q8h, CPK peaked (~ 3-4-fold above baseline) after 1 week and declined thereafter despite continued treatment

Microscopic Findings Indicative of Myopathy

Animals sacrificed at approximately one dosing interval after the last dose.

The incidence of muscle-related histopathological findings is presented as the number of sites affected divided by the number of sites examined. For skeletal muscle, 6 sites were examined in each of 4 dogs for a total of 24 sites.

Site / Lesion	Study A: Dose Regimen Saline q8h	25 mg/kg q24h	75 mg/kg q24h	25 mg/kg q8h	Study B: Dose Regimen Saline q8h	5 mg/kg q24h	5 mg/kg q8h
Skeletal muscle							
Myofiber degeneration	0/24	7/24	3/24	14/24	0/24	2/24	11/24
Myofiber regeneration	1/24	3/24	1/24	9/24	0/24	3/24	9/24
Diaphragm							
Myofiber degeneration	0/4	0/4	0/4	1/4	0/4	1/4	0/4
Heart							
Myofiber degeneration	0/4	0/4	0/4	0/4	0/4	0/4	0/4

Study A — Skeletal myofiber degeneration:

- Increased 2-fold at 25 mg/kg q8h vs 75 mg/kg q24h
- Increased 5-fold at 25 mg/kg q8h as compared to 25 mg/kg q24h
- Was of minimal severity, correlating to 3- to 25-fold increases in CPK

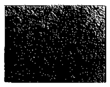
Study B — Skeletal myofiber degeneration:

- Increased 4-fold at 5 mg/kg q8h vs 5 mg/kg q24h
- Was of very minimal severity with very few fibers affected, correlating with 3- to 4-fold increases in CPK
- Was less severe in Study B than at higher doses used in Study A

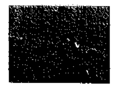
No degenerative effect on heart muscle at any regimen in either Study A or Study B

Histopathological Lesions in the Skeletal Muscle

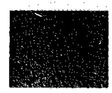
Photomicrographs of skeletal muscle sections at a total magnification of 64x



Treatment: saline (vehicle) q8h



Treatment: daptomycin at 5 mg/kg q24h
Note single degenerative fiber (arrow) with small numbers of inflammatory cells within the fiber basement membrane.



Treatment: daptomycin at 25 mg/kg q8h
Note degenerative fibers with inflammatory cell infiltrates (arrows). Regenerative fibers have smaller cross-sectional diameters, basophilic cytoplasm, and/or hypertrophied or centralized nuclei (arrowheads).
Note lack of repair by fibrosis.

Summary of Findings

Study A:

Dose Regimen	Total Daily Dose (mg/kg)	C_{max} (µg/mL)	AUC_{0-24} (µg·h/mL)	Peak CPK (IU/L)	Microscopic Myopathy[a]
saline q8h	0	0	0	315	0
25 mg/kg q24h	25	193	502	993	3
75 mg/kg q24h	75	549	1349	990	5
25 mg/kg q8h	75	233	2578	3000	15

[a]Number of skeletal muscle sites affected. Minimal degeneration in skeletal muscle sites, including diaphragm (7 sites in each of 4 dogs).

- At similar C_{max} values, a 3- to 5-fold increase in CPK levels and incidence of microscopic myopathy at 25 mg/kg q8h vs 25 mg/kg q24h
- At similar AUC_{0-24} values, a 3- to 4-fold increase in CPK levels and incidence of microscopic myopathy at 25 mg/kg q8h vs 75 mg/kg 24h. That is, dose fractionation increased toxicity
- Toxicity appears to be inversely related to time below a toxic plasma concentration. For example, time below 25 µg/mL C_{min} for 25 mg/kg q8h is 16, 12, and 8 hours for 25 mg/kg q24h, 75 mg/kg q24h, and 25 mg/kg q8h, respectively (see plasma concentration graph).

Study B:

Dose Regimen	Total Daily Dose (mg/kg)	C_{max} (µg/mL)	AUC_{0-24} (µg·h/mL)	Peak CPK (IU/L)	Microscopic Myopathy[a]
saline q24h	0	0	0	150	0
5 mg/kg q24h	5	58	180	150	7
5 mg/kg q8h	15	58	412	500	11

[a]Number of skeletal muscle sites affected. Minimal degeneration in skeletal muscle sites, including diaphragm (7 sites in each of 4 dogs).

- At a q24h dosing interval, the NOEL is approximately 5 mg/kg. This NOEL q24h results in no CPK changes and only very minimal histopathological evidence of skeletal muscle toxicity
- The NOEL q24h does not define a threshold plasma concentration for toxicity since administration every 8 hours (ie, 5 mg/kg q8h) leads to skeletal muscle toxicity evident by increases in CPK and microscopic myopathy
- Toxicity may be related to time below a given plasma concentration. For example, time below 10 µg/mL is 5 hours at 5 mg/kg q8h as compared to 18 hours at 5 mg/kg q24h (see plasma concentration graphs).

Conclusions and New Hypothesis

The pharmacokinetic parameter defining daptomycin-associated skeletal muscle toxicity in dogs:

- Is not related to C_{max}, AUC, or an intrinsically toxic plasma concentration
- Is related to dosing interval or percentage of time below toxic plasma concentrations

New hypothesis:

- Skeletal muscle toxicity is related to time between doses for repair of subclinical skeletal muscle damage. Once-daily dosing results in greater time for repair and, therefore, less toxicity

This new repair hypothesis is consistent with:

- The lack of progression of toxicity upon extended durations of dosing (as observed in 1- vs 6-month studies in the dog)
- The decrease in CPK levels despite continued treatment
- The presence of regenerative changes in the skeletal muscle

  Note: The observed relationship between dosing interval and toxicity is not unique to daptomycin. Once-daily dosing also decreases the nephrotoxicity induced by aminoglycosides (Barclay et al. 1994; Powell et al. 1983; Aronoff et al. 1983; and Periti 1995)

In addition, since C_{max} is the key determinant of efficacy in animal models of infection (Leggett et al. 1987), the pharmacological activity of daptomycin is optimized by once-daily dosing.

Thus, since safety and efficacy are not related to the same determinant, the safety margin for daptomycin can be increased by altering the dose regimen.

Impact on clinical trials:

- Development of daptomycin had been put on clinical hold as the clinical dose regimen was increased from 3 mg/kg q12h to 4 mg/kg q12h, based on the presumption that the resultant skeletal muscle toxicity was related to C_{max} or AUC
- Based on the new hypothesis, clinical trials have been reinitiated at dose regimens up to 6 mg/kg q24h
- Ongoing and future Phase 2 and 3 clinical trials will focus on a once-daily dosing regimen

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#244-SA

Effect of Oral Daptomycin on Vancomycin-Resistant *Enterococcus Faecium* (VREF) Gastrointestinal Tract Colonization in Antibiotic-Treated Mice

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Abstract

Daptomycin is a natural product lipopeptide antibiotic derived from *Streptomyces roseosporus*. It exhibits potent calcium-dependent bactericidal activity against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. The objective of this study was to examine the efficacy of daptomycin in eradicating vancomycin-resistant *Enterococcus faecium* (VREF) from a mouse model of gastrointestinal colonization. CD-1 female mice were pretreated with streptomycin (5 mg/ml) in their drinking water for 7 days, then inoculated with 7×10^8 cfu of a clinical isolate of VREF (ATCC 51559) by oral gavage. Persistent colonization of VREF ($>10^8$ cfu/g of feces) was established. Oral administration of daptomycin at 5 mg/mouse, twice daily for 7 days reduced VREF in the feces to below detectable levels (<10^3 cfu/g). VREF recolonized the feces of all animals (10^8 cfu/g) 7 days after discontinuation of daptomycin. These results show that oral administration of daptomycin at this dose effectively suppresses VREF in a murine model.

Introduction

Daptomycin is a natural product lipopeptide antibiotic derived from *Streptomyces roseosporus*. It exhibits potent calcium-dependent bactericidal activity against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). VRE infection has recently become a significant clinical problem. New medicines are needed for the treatment of this emergent disease. The present study examined the *in vivo* efficacy of daptomycin in the eradication of vancomycin-resistant *Enterococcus faecium* (VREF) colonization from the gastrointestinal tract of the mouse.

Objectives of the Study

To evaluate the *in vivo* efficacy of oral administration of daptomycin in a mouse model of VRE colonization of the gastrointestinal tract.

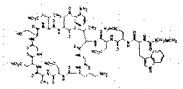
Daptomycin

Chemical Structure

Class of Drug: Lipopeptide (MW 1620, water soluble)

Possible Mechanisms of Action:

- Inhibits bacterial teichoic acid synthesis
- Disrupts membrane potential



Experimental Protocol

Animals: 20 female CD-1 mice, 20±2 g, divided into 4 groups

Pathogen: Clinical isolate of vancomycin-resistant *Enterococcus faecium* (ATCC 51559) was cultured in Brain Heart Infusion broth at 37°C for 18 h before the oral inoculation.

Fecal Bacterial Counts: Fresh feces were collected from individual mice for bacterial counts on the days specified. Colony forming units (CFU) of enterococci and VRE were determined by standard serial dilution on enterococcosel agar, and on enterococcosel agar containing 10 µg/ml of vancomycin, respectively.

Reference:

Anderson MS et al. Gastrointestinal tract colonization with vancomycin-resistant *Enterococcus faecium* in an animal model. Antimicrob Agents Chemother 1996; 40(6):1326-1330.

1. Establish Gastrointestinal Colonization of VRE

Day 0: Start streptomycin in drinking water (5 mg/ml) for group 3 and group 4; regular tap water for group 1 and group 2.

Day 6: Determine fecal CFU of total enterococci and VRE.

Day 7: Gastric inoculation of VREF 7×10^8 in 0.5 ml to all mice via gavage.

Day 13: Determine fecal CFU of total enterococci and VRE.

2. Eradicate Gastrointestinal Colonization of VRE with Oral Daptomycin

Day 14: Oral daptomycin 5 mg, b.i.d. to group 1 and group 3 for 7 days.

Day 19: Determine fecal CFU of total enterococci and VRE.

Day 21: Determine fecal CFU of total enterococci and VRE.

3. Examine Recurrence of Colonization

Day 21: Stop streptomycin in drinking water of group 3 and group 4.

Day 28: Determine feces CFU of total enterococci and VRE.

Table 1. Activity of Daptomycin and Vancomycin vs Gram-positive Pathogens* (Broth Microdilution Method, mg/l)

Organism	# of Strains	Agent	MIC_{90}	Range
S. aureus	20	daptomycin	0.25	0.06–1
	20	vancomycin	1	0.5–1
E. faecalis	20	daptomycin	1	0.25–2
	20	vancomycin	2	0.5–2
Vancomycin-resistant *Enterococcus* sp	20	daptomycin	2	0.5–2
	20	vancomycin	512	256–1024
S. pneumoniae	20	daptomycin	0.03	<0.0075–0.06
	20	vancomycin	0.25	0.015–0.5
S. pyogenes	-	daptomycin	0.25	0.015–0.25
	-	vancomycin	1	0.25–1

* Data shown are from 3 different studies conducted by Dr. David S. Goldstein of New England Medical Center, Boston, Mass.

Suppression of VRE by Daptomycin Treatment

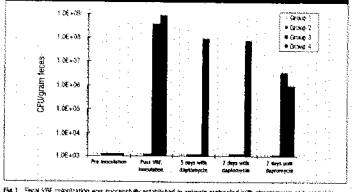


Fig 1. Fecal VRE colonization was successfully established in animals pretreated with streptomycin at 5 mg/ml in drinking water (group 3 and group 4). Daptomycin treatment for 5 and 7 days reduced fecal VRE from 10^8 cfu/g to undetectable levels (less than 1.3×10^3 cfu/g) in group 3 (group 4 is saline control). VRE colonization was not achieved in the absence of streptomycin pretreatment (group 1 and group 2). Fecal VRE colonization in group 3 recurred 7 days after discontinuation of daptomycin.

Suppression of Total Enterococci by Daptomycin Treatment

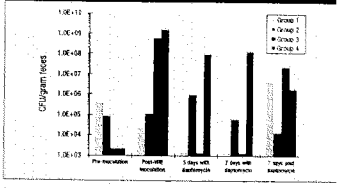


Fig 2. Fecal enterococcal CFUs were significantly increased in animals pretreated with streptomycin at 5 mg/ml in drinking water (group 3 and group 4). Daptomycin treatment suppressed fecal enterococci to undetectable levels (less than 1.3×10^3 cfu/g) in group 1 and group 3 (primarily VRE in group 3; see Fig 1). Group 2 and group 4 served as sham controls. Fecal enterococcal colonization in group 1 and group 3 recurred 7 days after discontinuation of daptomycin.

Animal Response to Daptomycin:

The dose of daptomycin was well tolerated by all of the animals treated. No abnormal symptoms or behaviors were observed during or after the antibiotic treatment.

Summary and Conclusion

1. Streptomycin in drinking water predisposed the mice to gastrointestinal colonization of VRE.
2. Daptomycin administered orally at 5 mg/mouse b.i.d. for 7 days effectively suppressed VRE colonization from the gastrointestinal tract of the animals.
3. A recurrence of VRE colonization in the gastrointestinal tracts of the mice was observed 7 days after daptomycin treatment was discontinued.

Effect of Daptomycin on Fecal Suspensions Seeded with a Vancomycin-Resistant Enterococcus

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Abstract

Vancomycin-resistant enterococci (VRE) have emerged as a significant nosocomial pathogen. The stools of colonized patients serve as the reservoir and mechanism for transmission. Control of carriers in hospitals is difficult. Daptomycin, a lipopeptide clinical development by Cubist Pharmaceuticals, Inc., has shown excellent in vitro inhibitory and bactericidal activity against VRE. Daptomycin tested in Ca^{++} supplemented MH broth at concentrations as low as 4 µg/ml reduced total bacterial counts to undetectable levels (<100 CFU/ml). We evaluated the antibacterial activity of daptomycin in fecal suspensions seeded with *E. faecium* (VRE; MIC/MBC of daptomycin, 1/2 mg/ml; MIC vancomycin 512 mg/ml). Pooled feces from normal volunteers were used to prepare fecal suspensions of 0%, 10%, 20%, 30%, 40%, and 50% (w/v) in Ca^{++} supplemented MH broth. Suspensions were seeded with approximately 10^8 CFU/ml of VRE. Effectiveness of daptomycin was determined by comparing the reduction in the CFU/ml from suspensions containing daptomycin (at 0, 25, 50, 100, 200, 400, and 800 µg/ml) to the CFU/ml from antibiotic- free cultures after overnight incubation at 37°C. In the fecal suspensions, a concentration-dependent killing could be observed for concentrations from 6-100 µg/ml. However, activity of daptomycin diminished as the concentration of feces in the medium increased. To reduce the CFU/ml to undetectable levels, 200 µg/ml of daptomycin was needed in a 10% fecal suspension, 400 mg/ml in a 20% suspension, and 400-800 µg/ml in a 30% suspension. With fecal suspensions of 40% and 50%, VRE inhibition could only be achieved until concentrations of daptomycin were 800 µg/ml. It appears that daptomycin is bound, inactivated, or metabolized by components in the feces, or that some other phenomena must be present that affect daptomycin's activity. Given the high concentrations achievable in the gut with oral administration, an oral formulation of daptomycin may hold promise in suppression of fecal carriage of VRE.

Introduction

Vancomycin-resistant enterococci (VRE) have emerged as significant nosocomial pathogens that account for approximately 8% of enterococcal infections in the U.S. Nosocomial infections with VRE are steadily increasing, especially among patients in intensive care. The stools of colonized patients serve as the reservoir and mechanism by which the organism is transmitted to other patients. Vancomycin resistance in enterococci poses a serious problem because it is usually accompanied by resistance to other antibiotics, such as penicillin and aminoglycosides, which are now rarely used for the treatment of this type of infection. Finding the availability of an effective therapeutic agent against VRE, the problem of carriers in hospitals is mainly controlled by isolating and restricting the patient from movement within the hospital and to extended-care facilities.

Daptomycin, a lipopeptide under clinical development by Cubist Pharmaceuticals, Inc., has shown excellent in vitro inhibitory and bactericidal activity against VRE. An oral formulation of daptomycin may hold promise in suppression of fecal carriage of VRE.

We studied the antibacterial activity of daptomycin in suspensions of normal human feces seeded with a vancomycin-resistant enterococcal strain. We also evaluated the following: supplementing the medium with calcium; the fecal concentrations of the suspensions; and the influence of the incubation environment on daptomycin.

Results

Experiment 1

Objective: To determine the effect of medium supplementation with Ca^{++} on unsupplemented medium and of incubation atmosphere (air vs anaerobic) on the antibacterial activity of daptomycin on *E. faecium* TAL 9867.

% FECES	DAPTOMYCIN MIC / ML	Unsupplemented MH + 1.25mM Ca^{++} Anaerobic 35C Incubation CFU / ML	Unsupplemented MH + 1.25mM Ca^{++} Aerobic 35C Incubation CFU / ML	Mueller Hinton Broth Anaerobic 35C Incubation CFU / ML	Mueller Hinton Broth Aerobic 35C Incubation CFU / ML
0%	0	1.2E+09	1.11E+08	2.42E+09	1.3E+09
0%	1	<100	<100	1E+07	6.5E+07
0%	2	<100	<100	5.8E+07	2.4E+07
10%	0	4E+04	4.3E+04	2.6E+04	8E+05
10%	1	1.1E+04	1.2E+04	1.4E+04	1.1E+05
10%	2	2.7E+04	1.7E+04	1.1E+04	1.9E+05
20%	0	7E+03	1.9E+04	6.9E+04	1.2E+04
20%	1	1.3E+04	1.9E+03	1.1E+04	1.1E+05
20%	2	1.9E+03	1.1E+04	1.5E+04	1.1E+05
30%	0	1.9E+03	1.3E+04	1.7E+05	2.6E+05
30%	1	1.7E+03	1.3E+04	1.4E+04	2.4E+05
30%	2	1.7E+03	1.2E+04	1.8E+05	1.7E+05
40%	0	1.6E+03	1.1E+04	2.9E+04	1.5E+04
40%	1	1.3E+03	1.7E+04	2.7E+04	4E+04
40%	2	1.7E+03	2.8E+04	2.3E+04	1.1E+04
50%	0	1.3E+03	1.7E+04	4.1E+04	1.7E+04
50%	1	2.1E+04	1.9E+04	2.8E+04	1.5E+05
50%	2	1.2E+03	9E+04	2.3E+04	1.8E+05

Daptomycin, tested in Ca^{++} supplemented MH at concentrations of 4 and 8 µg/ml reduced the total colony counts below detectable level of 100 cfu/ml. When tested in unsupplemented MH, daptomycin was virtually inactive in both concentrations.

The presence of feces alone (without antibiotic) in the test medium inhibited the growth of *E. faecium* TAL 9867; the cultures failed to grow beyond the initial inoculum. This effect was noticed in all fecal concentrations (10 - 50%). Daptomycin at 4 or 8 µg/ml failed to show additional antibacterial effect.

Incubation atmosphere did not affect the total bacterial counts; however, colonies grown anaerobically were larger and easier to read.

Experiments 2 and 3

Objective: To determine the antibacterial effect of higher concentrations of daptomycin vs *E. faecium* TAL 9867 seeded into fecal suspensions. Based on results from experiment 1, testing was done only in Ca^{++} supplemented MH and incubation under anaerobic conditions. Through the experiments, daptomycin was tested at higher concentrations of 200, 400, and 800 µg/ml. A concentration of 0 µg/ml was used as control.

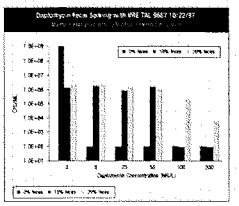
% FECES	DAPTOMYCIN MIC / ML	Experiment 2 Specimen 1,3 CFU / ML	Experiment 3 Specimen 4,7 CFU / ML
0%	inoculum	1E+09	1.92E+09
0%	0	2.12E+09	1.92E+09
0%	25	1.26E+07	1.38E+07
0%	50	1.05E+07	1.38E+07
0%	100	1.05E+07	1.38E+07
0%	200	1.05E+07	1.38E+07
0%	400	1.05E+07	1.38E+07
0%	800	not done	1.38E+07
10%	inoculum	1.8E+08	1.92E+08
10%	0	1.8E+08	1.92E+08
10%	25	1.8E+08	5.44E+05
10%	50	1.8E+08	5.44E+05
10%	100	1.8E+08	5.44E+05
10%	200	1.05E+07	1.38E+07
10%	400	1.05E+07	1.38E+07
10%	800	not done	1.38E+07
20%	inoculum	2.1E+08	2.16E+08
20%	0	1.8E+08	1.92E+08
20%	25	1.8E+08	1.92E+08
20%	50	1.8E+08	5.44E+05
20%	100	1.8E+08	5.44E+05
20%	200	1.05E+07	1.38E+07
20%	400	1.05E+07	1.38E+07
20%	800	not done	1.38E+07
30%	inoculum	1.8E+08	2.16E+08
30%	0	1.8E+08	1.92E+08
30%	25	1.8E+08	1.92E+08
30%	50	1.8E+08	5.44E+05
30%	100	1.8E+08	5.44E+05
30%	200	1.8E+08	5.44E+05
30%	400	1.05E+07	1.38E+07
30%	800	not done	1.38E+07
40%	inoculum	1.8E+08	2.16E+08
40%	0	1.8E+08	1.92E+08
40%	25	1.8E+08	1.92E+08
40%	50	1.8E+08	1.92E+08
40%	100	1.8E+08	1.92E+08
40%	200	1.8E+08	1.92E+08
40%	400	1.8E+08	1.92E+08
40%	800	not done	1.92E+08
50%	inoculum	1.8E+08	1.92E+08
50%	0	1.8E+08	1.92E+08
50%	25	1.8E+08	1.92E+08
50%	50	1.8E+08	1.92E+08
50%	100	1.8E+08	1.92E+08
50%	200	1.8E+08	1.92E+08
50%	400	1.8E+08	1.92E+08
50%	800	not done	1.92E+08

The antibacterial effect of daptomycin was considerably impaired by the fecal material in the medium. At a given inoculum, the amount of antibiotic needed to produce a reduction on the total bacterial counts was directly related to the amount of fecal material in the test medium. To reduce the counts below non-detectable levels, 200 µg/ml of daptomycin were needed in a 10% fecal suspension, 400 µg/ml in a 20% fecal suspensions, and 400-800 µg/ml in a 30% fecal suspension. A reduction equivalent to <100 cfu/ml was not achieved even at 800 µg/ml of daptomycin in 40% and 50% fecal suspensions.

Experiment 4

Objective: To determine for 90 min. slower, the concentration of daptomycin needed to produce a reduction on the total bacterial count equivalent to <100 cfu/ml in fecal suspension. Results from previous experiments leveled the fecal concentrations in the suspensions to 10% and 20%.

% FECES	DAPTOMYCIN MIC / ML	Specimen 10 CFU / ML
0%	inoculum	2.56E+09
0%	0	4.20E+09
0%	6	1.00E+02
0%	25	1.00E+02
0%	50	1.00E+02
0%	100	1.00E+02
0%	200	1.00E+02
10%	inoculum	1.00E+08
10%	0	3.00E+08
10%	6	3.00E+08
10%	25	1.80E+05
10%	50	1.10E+05
10%	100	3.00E+02
10%	200	3.00E+02
20%	inoculum	1.00E+08
20%	0	3.00E+08
20%	6	3.00E+08
20%	25	3.00E+08
20%	50	3.00E+08
20%	100	3.00E+08
20%	200	3.00E+02



Summary

- The study showed that daptomycin has potent antibacterial activity against VRE. A concentration-dependent killing was observed in both aerobic and anaerobic conditions.
- Daptomycin's in vitro activity is dependent on the presence of Ca^{++} in the test medium.
- The activity of daptomycin was greatly impaired by the presence of fecal material. This decreased activity was directly related to the concentration of feces in the suspensions.

Conclusions

- It appears that daptomycin may be bound, inactivated, or metabolized by fecal components, or that the calcium concentration in the fecal suspensions is affected nonetheless to enhance activity in the colonic milieu.
- If development of the antibiotic as an oral formulation is intended, problem of impaired activity of daptomycin in feces needs to be

In Vitro Activity of Daptomycin Against Resistant Gram-Positive Pathogens

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Abstract

Daptomycin is a lipopeptide antibiotic derived from the fermentation of *Streptomyces roseosporus*. Previously, daptomycin is undergoing clinical development by Cubist Pharmaceuticals, Inc. Studies published from 1986-1990 showed that daptomycin has excellent in vitro activity against resistant Gram-positive pathogens, such as methicillin-resistant *S. aureus* (MRSA), penicillin-resistant *S. pneumoniae* (PRSP), and vancomycin-resistant enterococci (VRE). In contrast to vancomycin and teicoplanin, daptomycin shows a bactericidal effect against staphylococci and enterococci. We evaluated the *in vitro* activity of daptomycin against 235 Gram-positive clinical isolates (27 methicillin-susceptible *S. aureus*; 54 MRSA; 22 penicillin-susceptible *S. pneumoniae*; 40 PRSP; 10 *S. pyogenes*; 20 vancomycin-susceptible enterococci; and 12 VRE) using NCCLS-recommended dilution techniques. Bactericidal activity was evaluated on selected organisms using MIC/MBC comparisons. The MIC range and MIC_{90} (µg/ml) for daptomycin against resistant organisms were: MRSA, 0.5 - 1, 1; PRSP, 0.06 - 1, 0.5; VRE 0.3 - 2, 2; whereas as values were MRSA 0.5 - 2, 2; PRSP 0.06 - 1, 0.25; VRE, ≤64. The results of our studies indicate that the MICs of daptomycin were, in general, two to four fold lower than those of vancomycin. The MBCs of daptomycin against VRE were, for the most part, within 1 - 2 dilutions of the MICs. Bactericidal activity of daptomycin was also demonstrated by time-kill curves against strains of VRE and MRSA. The excellent *in vitro* activity of daptomycin makes this drug a very good candidate for further clinical development against infections with resistant Gram-positive bacteria.

Introduction

Daptomycin is a somewhat hydrophobic antibiotic with excellent *in vitro* activity against Gram positive bacteria. Its spectrum of activity includes resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant, vancomycin-intermediately susceptible *Staphylococcus aureus* (VISA), *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *Enterococcus* spp. (VRE). A distinguishing characteristic of daptomycin over vancomycin and other glycopeptides is its concentration-dependent bactericidal activity against enterococci and staphylococci. Another unique feature of daptomycin is the requirement of ionized Ca^{++} in the test medium in order to exert its antibacterial activity. The antibiotic underwent clinical development during the late 1980s, but its development was discontinued. Recently, interest in this agent has been resurrected because of the problem of emergence of Gram-positive resistant pathogens against which there are very few, if any, effective antibiotics. The objective of this study was to compare the antibacterial activity of daptomycin to that of vancomycin against recently isolated Gram-positive resistant cocci and to demonstrate its bactericidal effect against vancomycin-resistant enterococci.

Materials and Methods

Bacterial isolates. All isolates were non-duplicated Gram-positive cocci. Forty PRSP and 50 MRSA were given to us, respectively, by Drs. J.R. Lonks and J.M. Boyce, Miriam Hospital, Providence, Rhode Island. The methicillin-resistant *Staphylococcus spp.* were all isolated during 1997-1998 at the clinical microbiology laboratory of MDMC. 79% (73/92) were blood isolates. Twenty-three *Enterococcus faecium*, vancomycin-resistant (VRE) strains used to determine the effect of Ca^{++} supplementation were confirmed by bullet-field gel electrophoresis as belonging to different clones. The following strains were tested:

• *Staphylococcus aureus* (MRSA)	54
• *Staphylococcus aureus* (MSSA)	27
• *Staphylococcus* spp. (MRSE)	79
• *Streptococcus pneumoniae* (PRSP)	40
• *Streptococcus pneumoniae* (Pen susceptible)	22
• *Streptococcus pyogenes*	10
• *Enterococcus faecium* (VRE)	33
• *Enterococcus faecalis* (Vanco-susceptible)	20
• Total isolates tested:	235

Determination of MICs for Staphylococci:

- Agar dilution using Mueller Hinton agar following NCCLS recommendations
- Antibiotic containing plates prepared freshly on the day of the test.
- Test concentration range for daptomycin and vancomycin was 32 - 0.06 µg/ml
- Test concentration range trovafloxacin and ciprofloxacin was 16 - 0.06 µg/ml
- Isolates grown in logarithmic phase in MH broth, turbidity adjusted to 0.5 MacFarland standard (~10^8 CFU/ml).
- Inocula delivered to the surface of the agar with a Steers replicator
- Inoculum density of ~10^4/spot
- MICs were read after 18 hours of incubation.
- *S. aureus* ATCC 29213 was used as control for MIC determinations.

Resistance to methicillin:

- MRSA confirmed with oxacillin salt agar plate (4% NaCl and 6 µg/ml of oxacillin)
- MRSE confirmed using an MIC breakpoint of ≥8 µg/ml as determined in a three years run
- *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300 were used as controls.

Determination of MICs for *S. pneumoniae* and *Enterococcus faecium*

- Broth microdilution following NCCLS recommendations.
- Mueller Hinton II manufacturer adjusted to concentrations of 20 - 25 mg/L of Ca^{++} and 10 - 12.5 of Mg^{++} (BBL, Cockeville, MD).
- Serial two-fold dilutions of the antibiotics in microtitre plates.
- The concentrations ranges were: daptomycin and vancomycin, 16 - 0.016 µg/ml; trovafloxacin and penicillin, 8 - 0.0078 µg/ml.
- *S. pneumoniae* grown overnight in 5% CHB chocolate broth (Remel, Lenexa, KS) and diluted in Mueller Hinton II (w 5% CHB =Horse blood-).
- *E. faecium* isolates were grown to logarithmic phase in MH II.
- Inoculum density ~ 10^4 CFU/well or ~ 5 x 10^5 CFU/well.
- Incubation in ambient air at 35°C for 20 - 24 and 18 hours, for *S. pneumoniae* and *E. faecium*, respectively.
- MICs were read as the lowest concentration of antibiotic that showed no turbidity or growth.

Determination of Bactericidal Activity:

Bactericidal activity was evaluated by two methods:

A) MIC/MBC

- Determined on 17 *E. faecium* (VRE).
- After MICs were read, aliquots of 0.01 ml of all wells with no visible growth were spread onto blood agar plates.
- Aliquots from the first two wells that showed growth (two dilutions below the MIC) were also plated to confirm the purity of the growth.
- The agar plates were incubated for 18 hours and colony counts were performed.
- MBCs within two dilutions of MICs were considered bactericidal.

B) Time-kill Curves: Performed with daptomycin concentrations equivalent to MIC, 4 MIC, and 8 MIC

- The bacteria used for these evaluations were:
  E. faecium TAL 3667 (VRE); MIC = 2 µg/ml
  S. aureus TAL 50 (MRSA); MIC = 1 µg/ml

- Bacterial cultures containing an inoculum in log phase of ~ 10^8 cfu/ml were mixed in corresponding concentration of the antibiotic and incubated (shaken) at 35°C in ambient air.
- Aliquots were withdrawn at 0, 15 minutes, 1 hour, 2 hours, 6 hours, and 24 hours.
- Diluted and plated onto MH agar, incubated at 35°C/18 - 20 hours in ambient air.
- Bactericidal activity was defined as 99.9% reduction of the total bacterial count as compared to the cfu/ml of the initial inoculum (t 0) done.

Determination of effect of increasing Ca^{++} concentration on the MICs of *E. faecium*:

- MICs of 23 *E. faecium* determined by broth microdilution in media with the following calcium concentrations*:
  1. Mueller Hinton broth "unsupplemented"
  2. MH II BBL, Cockeville, MD)
  3. MH + 25 mg/L Ca^{++}
  4. MH + 50 mg/L Ca^{++}
  5. MH + 75 mg/L Ca^{++} (5)
- The isolates were grown to MH II broth to logarithmic phase.
- Diluted to a density of ~ 10^4 CFU/mL inoculum of 5 x 10^4 CFU/well.
- Incubation in ambient air at 35°C for ~ 18 hours.
- MICs were read after ~ 18 hours incubation.

*Samples of the Ca^{++} supplemented broths MH II and MH were sent to the Chemistry Laboratory to determine the ionic and ionic calcium.

Summary

Daptomycin, a lipopeptide in the process of clinical development, showed excellent *in vitro* activity against susceptible and resistant Gram-positive pathogens.

- Its activity exceeded that of vancomycin against:
  Staphylococcus species
  MRSA, MSSA, MRSE (methicillin resistant coagulase negative Staphylococcus spp.)
  Enterococcus spp.
  Vancomycin-resistant *E. faecium* (VRE)
  Vancomycin- susceptible *E. faecalis*
  Streptococcus pneumoniae (penicillin susceptible) and *S. pyogenes*
- Daptomycin showed somewhat higher MICs than vancomycin against *Streptococcus pneumoniae* (PRSP).
- Daptomycin was bactericidal against 17 strains of *E. faecium* (VRE).
- Strains resistant to methicillin, vancomycin, or penicillin showed essentially the same MICs to daptomycin as those of susceptible strains, indicative of lack of cross resistance and/or different mechanism of action.
- The *in vitro* activity of daptomycin is highly dependent on the calcium concentration of the test medium? Calcium supplemented medium should be used to determine *in vitro* activity to daptomycin.

Conclusions

The *in vitro* activity of daptomycin against resistant Gram positive bacteria that include bactericidal activity against MRSA and VRE makes this antibiotic an excellent candidate for clinical development for the treatment of infections caused by these multiple resistant pathogens.

Table 1: Susceptibility of Gram-Positive Clinical Isolates to Daptomycin and Vancomycin

		Daptomycin	Vancomycin
S. aureus MRSA (54)	MIC_{50}	1	1
	MIC_{90}	1	1
	MIC Range	0.5 - 1	1 - 2
	% Resistant	0	0
S. aureus Meth-S (27)	MIC_{50}	0.75	0.5
	MIC_{90}	1	1
	MIC Range	0.06 - 1	0.5 - 2
	% Resistant	0	0
Staphylococcus spp. (Coag. Neg.) (79)	MIC_{50}	0.5	2
	MIC_{90}	1	4
	MIC Range	0.25 - 2	2 - 4
	% Resistant	0	0
S. pneumoniae (PRSP) (40)	MIC_{50}	0.5	0.25
	MIC_{90}	1	0.5
	MIC Range	0.06 - 1	0.06 - 1
	% Resistant	0	0
S. pneumoniae (Pen S) (10)	MIC_{50}	0.03	0.125
	MIC_{90}	0.06	0.25
	MIC Range	0.0015 - 0.06	0.015 - 0.5
	% Resistant	0	0
S. pyogenes (10)	MIC_{50}	0.015	0.25
	MIC_{90}	0.125	0.5
	MIC Range	0.0075 - 0.5	0.25 - 1
	% Resistant	0	0
E. faecalis (Vanco-S) (20)	MIC_{50}	1	1
	MIC_{90}	2	2
	MIC Range	1.25 - 2	0.5 - 2
	% Resistant	0	0
E. faecium (VRE) (23)	MIC_{50}	2	>64 - 512
	MIC_{90}	2	>64 - 512
	MIC Range	0.5 - 2	>64 - 1024
	% Resistant	0	100

*Tested concentration of vancomycin at least 4 times more than

1. Supplementation with calcium done using a $CaCl_2.2H_2O$ stock solution that contained 50 mg of Ca^{++}/ml.
2. Except for one experiment, the MICs reported on this study were determined in a medium containing less than 50 mg/l Ca^{++}, the concentration of Ca^{++} recommended by Cubist Pharmaceuticals, Inc.

Table 2: Bactericidal Activity of Daptomycin Against VRE*

Isolate #	MIC (µg/ml)	MBC (µg/ml)	MBC (µg/ml)
	Daptomycin	Daptomycin	Vancomycin
6458	0.5	1	>64
11405	1	1	>64
16357	1	1	>64
14977	1	1	>64
13628	1	4	>64
11814	1	4	>64
3733	1	4	>64
10018	1	4	>64
582	1	4	>64
6360	1	4	>64
4815	1	4	>64
3667	1	4	>64
5418	1	4	>64
606	2	4	>64
3817	2	4	>64
13855	2	4	>64
6373	2	4	>64

*All strains identified as *E. faecium*

Table 3: Susceptibility of Vancomycin-Resistant *Enterococcus* (VRE) on MH Broth

Supplemented with Various Concentrations of Calcium*

Isolate #	Baseline Values 0	25 mg/L Ca^{++}	50 mg/L Ca^{++}	75 mg/L Ca^{++}
10014	1	1.25	1.25	2.5
5418	1	2.5	2.25	5.0
3667	1	2.5	2.25	5.125
5418	1	2.25	2.25	5.125
6360	1	1.75	2.25	5.125
12513	1	1.25	2.25	5.125
5458	1	2.5	2.25	5.125
9667	1	1.25	2.25	5.125
1733	1	1.25	2.25	5.125
3817	1	2.5	2.25	5.125
4815	1	2.5	2.25	5.125
6458	1	2.5	2.25	5.125
10981	1	2.5	2.25	5.125
16835	1	2.5	2.25	5.125
14-10	1	2.5	2.25	5.125
582	1	2.5	2.25	5.125
3421	2	2.5	2.25	5.125
2433	2	2.5	2.25	5.125
3294	2	2.5	2.25	5.125
11575	2	2.5	2.25	5.125
566	2	2.5	2.25	5.25
17948	2	2.5	2.25	5.25
13214	1	2.5	2.25	5.125
6373	1	2.25	2.125	5.25
MEAN	1	2.5	2.125	5.125
MIC 90	1	2.5	2.25	5.25
MIC Range	1 - 2	2.25 - 2.5	2.125 - 2.25	5.25 - 5.25

*The MH broth lacked concentrations of Ca^{++} was prepared following instructions outlined by NCCLS Document M7-A4 Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically - Fourth edition; Approved standard - 1997 - pp 12 - 3. See note below.
Testing conditions:
Medium: Mueller Hinton broth supplemented with indicated concentrations of Calcium.
Inoculum: Grown to logarithmic phase in tube of broth, diluted to ~ 5 x 10^4 cfu/well
Incubation: ambient air, 35°C - 18 hours

Table 4: Calcium Concentrations by Chemical Analysis of the Various MH test broths

Medium	Total Calcium (mg/L)	Ionic calcium (mM)
MH*	23	1.22
MH II	23	1.26
MH + 25 mg/L Ca^{++}	46	1.92
MH + 50 mg/L Ca^{++}	66	3.3
MH + 75 mg/L Ca^{++}	88	3.5

*Media labeled MH, no calcium supplementation indicated. Laboratory prepared media was unsupplemented and used it as the base medium to prepare the supplemented media. Base broth of calcium. It was found that it contains a calcium concentration within the range of that in the medium labeled Mueller Hinton II (~ 23 mg/L). This explains the higher than expected concentrations of calcium in the experimentally prepared MH broths.

Fig. 1-Bactericidal Activity of Daptomycin Against MRSA #50

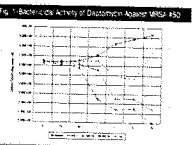
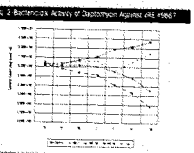


Fig. 2 Bactericidal Activity of Daptomycin Against VRE #5667



#16406

In Vivo Efficacy of Daptomycin Against Systemic Infection Induced by Vancomycin-Resistant *Enterococcus Faecalis* (VRE) in the Mouse

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Abstract

Daptomycin is a natural product lipopeptide antibiotic derived from *Streptomyces roseosporus*. The drug acts on a unique cell wall target, likely lipoteichoic acid. Daptomycin exhibits potent bactericidal activity in vitro against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. Preliminary animal studies showed that daptomycin administered subcutaneously (s.c.) at 2 mg/kg protected all the mice from systemic infection with VRE. The objective of this study was to establish the 50% protective dose (PD_{50}) of daptomycin in a mouse model of systemic infection induced by a clinical isolate of VRE (#80, daptomycin MIC 2-3 µg/ml). Five groups of CD-1 female mice were inoculated intraperitoneally with a lethal dose of VRE #80 ($1x10^8$ cfu/mouse). Immediately after the bacterial inoculation, control animals were given saline or vancomycin 50 mg/kg s.c. Daptomycin was administered s.c. in doses of 5.0, 1.0, and 0.2 mg/kg. Injections of saline, daptomycin and vancomycin were repeated once, 4 hours after the inoculation. The PD_{50} of daptomycin against the systemic VRE infection in the mouse was calculated by the Method of Probits to be 1.2 mg/kg, based on the number of mice surviving 7 days after the inoculation. All mice treated with saline and vancomycin died within 3 days after the inoculation. The results of this study suggest that daptomycin is an effective therapeutic against the mouse systemic infection caused by VRE.

Introduction

Daptomycin is a natural product lipopeptide antibiotic derived from *Streptomyces roseosporus*. The drug acts on a unique cell wall target, likely lipoteichoic acid. It exhibits potent bactericidal activity in vitro against Gram-positive organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). VRE infection has recently become an urgent clinical problem while no FDA-approved medicines are available for the emerging disease. The present study examined the *in vivo* efficacy of daptomycin against a systemic infection experimentally induced by VRE.

Objectives of the Study

1. To evaluate the antibacterial efficacy of daptomycin in a mouse model of systemic infection induced by vancomycin-resistant *Enterococcus faecalis* (VRE).
2. To establish the 50% protective dose (PD_{50}) of daptomycin in the mouse model of infection.

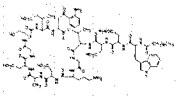
Daptomycin

Chemical Structure

Class of Drug: Lipopeptide (MW 1620, water soluble)

Possible Mechanisms of Action:

- Inhibit bacterial lipoteichoic acid synthesis
- Disrupt membrane potential
- Calcium-dependent



Experimental Procedures

1. Animals: CD-1 female mice (Charles River Lab, MA) weighing 18-23 g were used in this study. There were 5 mice in each group. Water and Agway rodent chow were provided *ad libitum* throughout the study.
2. Pathogen used to induce infection was a clinical isolate of VRE (#80) obtained from New England Medical Center. The bacterial strain was cultured in Brain Heart Infusion (BHI) broth (BBL, MD) at 37°C for 18 hours, and then concentrated 20 fold by centrifuge (8000 rpm for 15 minutes).
3. Virulence titration of VRE: 50 µl of the concentrated overnight culture of VRE was diluted to 1.0 ml with the medium, read their OD_{650} of 0.8-1.2 (BHI medium as blank). To compare the inocula, 1.0 ml of the concentrated culture was diluted to 8 ml with 0.01 M PBS (Sigma # 0261) containing 5% hog gastric mucin (Sigma M-2378). Two inoculum ($1.6x10^8$ cfu/ml) was further diluted to $3x10^7$, $1x10^7$, and $3.1x10^6$ cfu/ml with the 5% mucin solution. Each inoculum was vortexed for 30 seconds. Six groups of mice were used for the virulence titration. The control group (Group 1) received 0.5 ml of the 5% mucin injection only. The mice in Groups 2, 3, 4, and 5 were intraperitoneally (i.p.) inoculated with an inoculum of VRE at $1.6x10^8$, $3x10^7$, $1.0x10^7$, $3.1x10^6$, and $1.4x10^6$ cfu/mouse, respectively, in a volume of 0.5 ml. Group 6 mice received the VRE at $1.4x10^6$ cfu/mouse and 2 doses of Daptomycin (dissolved in saline) at 50 mg/kg, s.c., immediately after inoculation, and 4 hours after inoculation.
4. Mouse Protection Test: 25 µl of the overnight-isolated overnight culture of VRE was diluted to 1.0 ml with BHI. Its OD_{650} of 0.783-1.894 (BHI medium as blank). The turbidity of a 0.5 McFarland standard is equivalent to OD_{650} 0.1 - $1.0x10^8$ cfu/ml. To compare the inocula, $4x10^{7.5}$ cfu (0.32 ml) from the concentrated culture was diluted to 20 ml with 0.01 M PBS containing 5% hog gastric mucin. Six groups of mice were used for the mouse protection test. All animals were injected with 0.5 ml of the inoculum (a volumetric $1.13x10^7$ cfu/mouse).

   Ten mg of daptomycin was dissolved in 20 ml of normal saline to give a solution of 0.5 mg/ml. This solution was serially diluted with saline by 5-fold (1 ml to 5 ml) to give 0.1 and 0.02 mg/ml solutions. The known antibiotics used as comparative controls were vancomycin (U.Signa V-2002, dissolved in saline) and Ciprofloxacin (Bayer 87-150, dissolved in water). 25 mg of each compound was dissolved in 5 ml vehicle to bring a solution of 5 mg/ml (pH=7.0). All the solutions were filtered through a 0.2 µm Nalgene syringe filter, immediately after the bacterial inoculation. Group 1 animals were s.c. injected with saline at 10 ml/kg (no antibiotic) and Groups 2 to 4 were given daptomycin s.c. at 5, 1, and 0.2 mg/kg, respectively. Group 5 animals received vancomycin s.c. at 50 mg/kg and Group 6 animals received Ciprofloxacin s.c. at 50 mg/kg. The injection volume for all the animals were 10 ml/kg. The same injections of saline, daptomycin, vancomycin, and Ciprofloxacin were repeated once 4 hours after the inoculation. The 50% protective dose (PD_{50}) of daptomycin against the systemic VRE infection in the mouse is calculated by the Method of Probits, on the basis of the number of mice surviving 7 days after inoculation.

Table 1. Gram-Positive MICs for Daptomycin and Vancomycin* (Broth Microdilution Method, mg/L)

Organism	# of Strains	Agent	MIC_{90}	Range
S. aureus	20	Daptomycin	0.25	0.06–1
	20	Vancomycin	1	0.5–1
E. faecalis	20	Daptomycin	1	0.25–2
	20	Vancomycin	2	0.5–2
Vancomycin resistant enterococci sp.	20	Daptomycin	2	0.5–2
	20	Vancomycin	>128	256–1024
S. pneumoniae	20	Daptomycin	0.03	<0.0015–0.06
	20	Vancomycin	0.25	0.015–0.5
S. pyogenes	7	Daptomycin	0.25	0.015–0.25
	7	Vancomycin	1	0.25–1

* Data is internal study conducted by Dr. Frank E. Nightingale at New England Medical Center, Boston.

Table 2. Results of Virulence Titration with VRE #80[1]

Group	Treatment	Inoculation (cfu/mouse)	Antibiotic (mg/kg)	Mortality (7 days)
1	Control (5% mucin 0.5 ml)	0	0	0/5
2	VRE #80 ip	$1.6x10^8$	0	0/5
3	VRE #80 ip	$3.0x10^7$	0	0/5
4	VRE #80 ip	$1.0x10^7$	0	1/5
5	VRE #80 ip	$3.4x10^6$	0	1/5
6	VRE #80 ip	$1.4x10^6$	0	5/5
	+ Daptomycin s.c.	$1.4x10^6$	50	0/5[2,3]

1. Clinical isolate of vancomycin-resistant *Enterococcus faecalis* with MIC to daptomycin 2-3 ug/ml.
2. Mortality records that remained stable on body weight was during the experiment.
3. Physical conditions and body weights of the mice were normal in survivors.

Summary of the Virulence Titration:

1. The 100% lethal dose of VRE #80 was $3.4x10^6$ cfu/mouse.
2. Daptomycin at 50 mg/kg s.c. protected all the mice from systemic infection induced by the VRE.

Table 3. Results of the Mouse Protection Test

Group	# of mice	Inoculation	Treatment	Survival (7 days)
1	5	VRE #80, $1x10^8$ cfu/mouse, ip	Normal saline[1] 10 ml/kg, s.c. x2	0/5
2	5	VRE #80, $1x10^8$ cfu/mouse, ip	Daptomycin[2] 5 mg/kg, s.c. x2	5/5
3	5	VRE #80, $1x10^8$ cfu/mouse, ip	Daptomycin 1 mg/kg, s.c. x2	1/5
4	5	VRE #80, $1x10^8$ cfu/mouse, ip	Daptomycin 0.2 mg/kg, s.c. x2	0/5
5	5	VRE #80, $1x10^8$ cfu/mouse, ip	Vancomycin[3] 50 mg/kg, s.c. x2	0/5
6	5	VRE #80, $1x10^8$ cfu/mouse, ip	Ciprofloxacin[4] 50 mg/kg, s.c. x2	0/5

1. Negative control
2. MIC of Daptomycin vs. VRE #80 was 2-3 ug/ml.
3. Comparative control: MIC >100 ug/ml vs. VRE #80.
4. Comparative control: MIC >100 ug/ml vs. VRE #80.

Table 4. PD_{50} Determination of Daptomycin in the Mouse Protection Test[1]

Date: 4/29/98				(by the Method of Probits)			
Daptomycin Dose (mg/kg, sc, BID)	log Dose	# of Mice	# Survivors	Protective %	Probit	Expected Probits	Expected
Saline (10 ml/kg)		5	0	0			
0.2	-0.70	5	0	0	2.674	3.262	0
1	0.00	5	1	20	4.158	4.113	38
5	0.70	5	5	100	7.228	7.045	37

* All the mice were inoculated with VRE #80 at 10^8 cfu/mouse ip on 4/22/98.

log PD_{50} = 0.084	
PD_{50} = 1.2	
SE of PD_{50}	R = 0.975
	Slope = 3.326
	Y Inter. = 4.719

95% confidence limits of the PD50 = 0.8 – 1.8 mg/kg

Fig. 1-PD_{50} of Subcutaneous Daptomycin

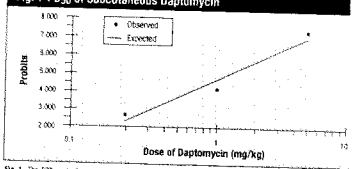


Fig. 1. The 50% protective dose (PD_{50}) of daptomycin against the systemic VRE infection in the mouse is calculated to be 1.2 mg/kg with 95% confidence limits of 0.8-1.8 mg/kg by the Method of Probits (the data are shown in Table 3).

Summary and Conclusion

1. Daptomycin, in a dose-dependent manner, protected the mice from systemic infection induced by vancomycin-resistant *Enterococcus faecalis* (VRE).
2. The results of this study suggest that daptomycin may be used as an effective therapeutic against systemic infection caused by VRE.

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In Vitro Studies on Resistance to the Lipopeptide Antibiotic Daptomycin

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Abstract

Daptomycin is a novel lipopeptide antibiotic with potent calcium-dependent bactericidal activity against a broad range of Gram-positive pathogens. The in vitro emergence of resistance to daptomycin has been studied using three methods: spontaneous resistance incidence (RI), serial passage in the presence of increasing drug concentrations, and chemical mutagenesis. No spontaneously resistant mutants were obtained for any organisms tested (RI <10^{-9} for *S. aureus*, <10^{-8} for *S. epidermidis*, <10^{-9} for *E. faecalis*, <10^{-7} for *E. faecium*). We demonstrate that daptomycin susceptibility is heterogeneous and that the extent of heterogeneity is sensitive to calcium concentrations and inoculum density. Both can affect apparent RI values and the reports of higher rates of resistance may reflect these factors. Stable *S. aureus* mutants with increased MICs have been achieved by serial passage in liquid broths and by chemical mutagenesis. MICs of the mutants are 8-16-fold higher than those of the parental strain. Mutants with high (> 11.5 μg/ml) MICs have significant growth defects but are still display phenotypes typical of *S. aureus* smooth colony variants. Overexpression of a 29kD protein was observed in these strains. Its identity and possible role in resistance is under investigation. It has been suggested that daptomycin alters the electrical component (ΔΨ) of the bacterial membrane potential (Δμ). We have developed an assay that demonstrates a reduction in ΔΨ following daptomycin addition in the mutants, accounting for their higher MIC.

Daptomycin

Daptomycin is a novel lipopeptide antibiotic produced by *Streptomyces roseosporus*. It has potent calcium-dependent bactericidal activity against Gram-positive pathogens including methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Daptomycin will enter clinical trials in 1999. Determination of spontaneous resistance rates and analysis of the behavior of strains challenged with sub-lethal levels of antibiotic is an important aspect of the preclinical evaluation of this compound.

The mechanism of action of daptomycin remains poorly defined. Interaction with cell membranes is mediated by the antibiotic's acid chain; specificity for Gram-positive bacteria may be conferred by interaction with specific target proteins. Previous research has suggested two likely targets: synthesis of the essential cell surface polymer lipoteichoic acid, or disruption of the ΔΨ component of the bacterial membrane potential. The possible utility of mutants in elucidating the mechanism of action and identifying targets for future screening and drug discovery prompted us to isolate and analyze resistant organisms.

Resistance: Spontaneous Incidence

Species	Strain #	MIC
S. aureus	12 (ATCC 29778) 16 (MRSA) 52 (MRSA)	>8 (>11.7) >8 (>11.7) >8 (>11.7)
S. epidermidis	1 2 101 113	>8 (>11.7) >8 (>11.7) >8 (>11.7) >8 (>11.7)
E. faecalis	201 19 30 (VRE)	>8 (>11.7) >8 (>11.7) >8 (>11.7)
E. faecium	1 297 (VRE)	>8 (>11.7) >8 (>11.7)
S. pneumoniae	13 14	>1 (>1.5) >1 (>1.5)

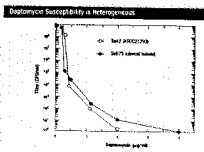
* Values are reported as [μg/ml] (mM)
** All tested isolates are wild-type

Rates of spontaneous resistance in laboratory RI were determined for the impervious Gram-positive pathogens. Laboratory and clinical isolates were selected by plating saturated overnight cultures on Mueller-Hinton agar (MHA) containing daptomycin at 4x the broth MIC.

Conclusion:

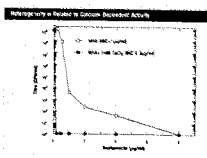
Spontaneous resistance to daptomycin is extremely rare in Gram-positive pathogens.

Daptomycin Susceptibility is Heterogeneous



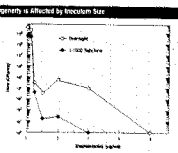
- Susceptibility to antibiotics may be homogeneous or heterogeneous (i.e., isolates may be recovered from cultures dosed at antibiotic concentrations at, or above, the MIC). In the investigations of Dr. Dana Haller, Wayne State University, we examined the nature of daptomycin susceptibility by titration of wild experimental cultures in MHA containing daptomycin.
- As shown above, daptomycin susceptibility is heterogeneous in laboratory and clinical isolates. Outcomes recovered at concentrations above the MIC are labelled 'resistant' and have MICs identical to parental strains following purification and retesting.
- Previous investigators have reported higher % values for daptomycin heterogeneity may lead to high % outcomes in the presence of increasing, if subtle.

Heterogeneity is Related to Calcium Dependent Activity



- The action of daptomycin is dependent on the presence of physiological levels of calcium in the test media.
- As demonstrated here, susceptibility to daptomycin is heterogeneous in the presence of 50mM $CaCl_2$.
- The basis of heterogeneous susceptibility may be related to the calcium-dependent step of daptomycin action.
- Low levels of calcium may result in artifactually high RI values.

Heterogeneity is Affected by Inoculum Size



- Previous investigators have reported more favorable effects for MIC testing of daptomycin.
- We have determined significant inoculum effects in RI testing.
- As demonstrated here, differences in culture density (~10-fold on MHA) result in 16-fold differences in *S. aureus* daptomycin RIs.
- Colonies recovered from plates containing >1μg/ml daptomycin are falsely resistant and have MICs identical to parental strains following purification and retesting.

Resistance: Serial Passage

- Because of their potential utility in understanding the mechanism of daptomycin action, resistant mutants were isolated using serial passage techniques.
- *S. aureus* and *E. faecalis* laboratory strains were serially passaged in Mueller-Hinton broth (MHB) containing increasing concentrations of daptomycin, starting at 1/4 MIC and continuing until three successive generations showed no increase in MIC. Mutants were purified by passage on MHA in the absence of daptomycin.
- *S. aureus* mutant CB270 has an MIC 16 fold higher than the parent strain, daptomycin remains bactericidal.
- CB270 was cross-resistant to the peptide antibiotic nisin but showed no significant MIC changes for other antibiotics.
- CB270 displays WT growth and virulence.
- *E. faecalis* mutants CB312 and CB313 have an MIC 32 fold greater than the parent.
- CB312 and CB313 demonstrated no cross-resistance to other tested antibiotics.

Resistance: Chemical Mutagenesis

- Isolation of mutants via serial passage lowers the outcome towards mutants displaying parental growth rates.
- In order to obtain a wider variety of mutated phenotypes, we chemically mutagenized *S. aureus* followed by direct selection for survival on MHA containing 2, 4, or 8 μg/ml daptomycin.
- Eleven independent mutants were isolated, displaying varied colony morphology and growth phenotypes.
- All mutants were verified as *S. aureus* by measuring coagulase activity and by PCR amplification using strain specific primers.
- Mutants fall into three classes.
- Class 1: Normal growth rate, virulence (mice) similar to wild (MIC = 100μg/ml). Serial passage mutant CB270 was classified with this group.
- Class 2: Reduced growth rate on MHA, poor or no growth in complete defined media (CDM), no antibiotic cross resistance.
- Class 3: Severe growth defects in MHB and CDM, pinpoint or non-pigmented on MHA, do not display classic small colony variant phenotypes; no antibiotic cross resistance.

Characterization of Chemically Induced Mutants: Growth Phenotypes

Class	Clones	Liquid Media (Hours)		Solid Media (Colony Size)		
		MHB	CDM	MHA	7.5% NaCl	SCV
247	WT	1.00	1.0	+++	+++	-
270	1	1.10	1.2	+++	+++	-
313	1	1.07	1.18	+++	+++	-
249	1	1.00	NG	+	+	-
267	1	1.05	NG	+	+	-
283	1	1.08	1.16	+++	+++	-
284	1	1.06	1.11	+++	+++	-
286	1	0.98	1.43	+++	+++	-
288	1	1.01	NG	+	+	-
291	1	1.21	NG	+	+	-
292	1	1.07	NG	+	+	-
293	1	1.10	NG	+	+	-
295	1	1.15	NG	+	+	-

Legend: MHB: Mueller-Hinton broth; CDM: Complete Defined Media; MHA: Mueller-Hinton agar; NG: No Growth; >8 hours; SCV: Detailed Growth characteristic at 24 hours); SCV: MHA plus hemin, thiamine, and menaquinone.

Conclusion:

At least three classes of mutants exist, based on growth phenotypes. Poorly growing mutants (Class 2 and 3) are not recovered by SCV supplements, supporting they are not small colony variants. Results from CDM support that not all Class 1 mutants display the same auxotrophies.

Characterization of Chemically Induced Mutants: MICs

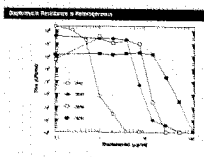
CB#	Class	MIC (μg/ml)				
		Oxa/a	Vanc	Rifa	Amik	Cipro
247	WT	0.78	0.78	0.25	12.5	0.78
270	1	12.5	1.56	0.25	>100	0.78
313	1	6.25	1.56	0.25	>100	0.78
249	1	6.25	3.13	NG	>100	0.39
267	1	6.25	1.56	NG	>100	0.39
283	1	3.25	1.56	0.25	>100	0.39
284	1	6.25	0.78	NG	>100	0.78
286	1	12.5	2.39	NG	>100	NG
288	1	12.5	2.39	NG	NG	1.56
291	1	25	3.34	1.56	>100	0.78
292	1	12.5	0.78	NG	NG	0.78
293	1	12.5	1.56	NG	NG	1.56
295	1	12.5	1.56	0.25	0.25	<0.1

Legend: Oxa/a: Oxacillin/acid; Vanc: vancomycin; Rifa: rifampicin; Cipro: ciprofloxacin; NG: not determined. All MICs were determined using NCCLS standard methods with a maximum tested concentration of >100μg/ml.

Conclusion:

Mutant classes, as assigned by growth phenotype, correlate with antibiotic susceptibility. Class 1 mutants were cross resistant to the peptide antibiotic nisin; no other cross resistance was observed. Class 2 and 3 mutants showed normal or increased susceptibility to gentamycin, again supporting the idea that they are not classical small colony variants.

Daptomycin Resistance is Heterogeneous

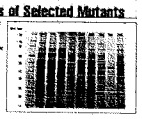


- Class 1 (CB267), Class 2 (CB295), and Class 3 (CB291) mutants tested. The latter expresses susceptibility of the parental strain (CB247).
- Mutants display homogeneous susceptibility in the presence of 50mM $CaCl_2$; blots not shown.

Daptomycin: Possible Mechanisms

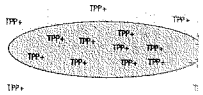
- The mechanism of action of daptomycin remains poorly defined. The target is likely to be in the cytoplasmic membrane as daptomycin can insert into liposomes but does not penetrate to the cytoplasm of target cells. Previous investigators have focused on two areas.
- Other cell membrane have demonstrated that daptomycin disrupts the electrical component (ΔΨ) of the bacterial membrane potential but does not affect (pH). Data have also demonstrated that the value of ΔΨ affects the activity of daptomycin.
- Consistent with this mechanism, have suggested that inhibition of lipoteichoic acid (LTA) biosynthesis is the target of daptomycin action and have demonstrated the accumulation of LTA precursors in daptomycin treated cells. There have also identified putative daptomycin binding proteins in the cytoplasmic membrane.
- We have supplemented selected daptomycin resistant mutants to determine if evidence in support of either model can be obtained.

Protein Profiles of Selected Mutants



- Whole cell extractions from overnight cultures were analyzed by SDS-PAGE on 4-20% gradient gels.
- No significant differences are visible in the protein profile of Class 1 mutants (CB270, 284, 287, 288).
- Class 2 and 3 mutants (CB283, 290, 291, 295) overexpress a 29kD protein (arrow).
- Further analysis by 2-dimensional electrophoresis and cell fractionation is underway.

ΔΨ is Increased in Class 1 Mutan



ΔΨ was measured by steady state accumulation of tetraphenylphosphonium bromide

Strain	Time (s)	ΔΨ (mV)	Time (s)
CB247		-173.5	-177
CB270		-167.7	-174
CB294		-148.5	-172
CB287		NG	-141

NG = no growth

- Class 1 mutants have an increased electrical potential across the cytoplasmic membrane.
- Others, et al, have demonstrated a reduction in ΔΨ following daptomycin addition to growing cultures.
- Higher levels of daptomycin may be required to fully dissipate the membrane potential in the mutants, accounting for their higher MIC.
- ΔΨ in Class 2 and 3 mutants is under examination.

Summary/Conclusions

- Spontaneous resistance to daptomycin occurs at very low rates (<10^{-9} for *S. aureus*, <10^{-7} for *E. faecalis* and *E. faecium*).
- Daptomycin susceptibility is heterogeneous, heterogeneity is influenced by calcium, inoculum and incubation density, and may affect RI values.
- Mutants with stable increased MICs (8-32X) were isolated by serial passage with sub-lethal levels and chemical mutagenesis.
- The mechanism of resistance in these isolates has not been determined.
- Based on growth phenotypes, three classes of mutants have been described.
- Overexpression of a 29kD protein is associated with resistance in slow-growing mutants (Class 1, 3).
- Membrane potential is altered in Class 1 mutants.

Future Directions

- Further analysis of 29 kD overexpressed protein from mutant
- Identification of overexpressed 29kD protein, determination of association with resis
- Further analysis of membrane potential in class 2 and 3 mutants.
- Analysis of LTA synthesis and structure in mutants.
- Isolation of mutants from additional species to determine if resistance mechanisms a

Cubist Pharmaceuticals Announces Additional Positive Safety and Efficacy Data on Cidecin(TM) (daptomycin) at ICAAC 2000

Invited Speaker, Dr. David Snydman, Presents Data on New Phase I Dose Escalation Trial and Phase II Studies

CAMBRIDGE, Mass., Sept. 19 /PRNewswire/ -- Cubist Pharmaceuticals, Inc. (Nasdaq: CBST) announced the presentation of positive safety and efficacy data on its novel investigational antibiotic Cidecin(TM) (daptomycin for injection) yesterday at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC 2000). David R. Snydman, MD, Professor of Medicine at Tufts University School of Medicine and Chief of Geographic Medicine and Infectious Disease at the New England Medical Center, discussed the characteristics of daptomycin and presented new clinical data as part of Monday afternoon's session on Emerging Therapies.

(Photo: http://www.newscom.com/cgi-bin/prnh/20000717/CUBELOGO)

Most notable were data presented on a recently completed Phase I daptomycin dose escalation study intended to assess the safety, tolerability and pharmacokinetics of daptomycin given once a day at increasing doses. In the study, volunteers received once-daily doses of daptomycin at 4 mg/kg, 6 mg/kg or 8 mg/kg for up to 14 days. The results of the study showed that no serious adverse events were attributable to the use of daptomycin in any patients at any of the doses studied.

Francis P. Tally, MD, Cubist's Executive Vice President for Scientific Affairs commented, "In our current Phase III clinical trials in skin and soft tissue infection, community-acquired pneumonia and complicated urinary tract infection, as well as in our ongoing Phase II study in bacteremia, daptomycin is being dosed at 4 mg/kg once daily. We felt it important to complete the Phase I dose escalation trial not only to present a more comprehensive safety package to the FDA, but also to facilitate additional planned clinical trials, in indications such as endocarditis and osteomyelitis, should higher dosing be necessary. We are very pleased with the results of the study and look forward to expanding our clinical experience with Cidecin."

In addition to the Phase I data, Dr. Snydman presented additional patient data from Cubist's two ongoing Phase II studies. The first study is focused on patients diagnosed with bacteremia, a serious bloodstream infection. The second study is focused on patients who have failed or are unable to tolerate other therapies for the treatment of serious Gram-positive infections, including bacteremia, complicated skin and soft tissue infection, complicated urinary tract infection, intra-abdominal infection and pneumonia. Combining the data from the 4 mg/kg once-daily dosing regimen (see Table 1), daptomycin had an overall clinical success rate of 93% in the modified intent-to-treat population and 100% on the clinically evaluable patients. In terms of microbiologic eradication, daptomycin demonstrated a 75% success rate in the modified intent-to-treat population and a 100% success rate in microbiologically evaluable patients. Comparable vancomycin data in the bacteremia trial demonstrated a 64% clinical success rate in the modified intent-to-treat population. These additional data presented are consistent with the data announced earlier this year; Cidecin appears to be efficacious in the treatment of bacteremia and other serious, life-threatening infections.

"As our experience with the use of daptomycin grows, I am pleased with its clinical efficacy and good safety profile," Dr. Snydman said. "Importantly, the data emerging from the daptomycin clinical trials continue to show promise to the medical community at a time when the need for novel antibiotics is escalating as a result of the increased bacterial resistance seen worldwide."

Cidecin is the first in a new class of antibiotics that has demonstrated rapid bactericidal activity against a wide range of Gram-positive bacteria, including strains resistant to current therapies. Cidecin is being developed to treat serious and life-threatening infections in hospitalized patients. Multiple, global Phase III EDGE(TM) (Evaluation of Daptomycin in Gram-positive

Entities) trials are currently underway investigating Cidecin's efficacy in the treatment of complicated skin and soft tissue infections (EDGESST). Cidecin is also involved in two, open-label Phase II studies-one for the treatment of bacteremia and another for the treatment of bacterial infections, including endocarditis, osteomyelitis, complicated urinary tract infection (cUTI), intra-abdominal infection and pneumonia, in patients who are resistant, refractory or contraindicated (RRC) to other therapies. Cubist is currently initiating Phase III trials in both cUTI (EDGEUTI) and community-acquired pneumonia (EDGECAP).

Cubist Pharmaceuticals is focused on becoming a global leader in the research, development and commercialization of novel antimicrobial drugs to combat serious and life-threatening bacterial and fungal infections. Cubist is evaluating the safety and efficacy of Cidecin(TM) (daptomycin for injection) in the EDGE(TM) (Evaluation of Daptomycin in Gram-positive Entities) clinical trial program and is engaged in multiple, strategic partnerships, including Novartis Pharma AG and Merck & Co. for the discovery and development of novel antiinfectives.

Cubist Safeharbor Statement

Statements contained herein that are not historical fact may be forward-looking statements within the meaning of Section 27A of the Securities Act of 1933 and Section 21E of the Securities Exchange Act of 1934, that are subject to a variety of risks and uncertainties. There are a number of important factors that could cause actual results to differ materially from those projected or suggested in any forward-looking statements made by the Company. These factors include, but are not limited to: (i) the Company's ability to successfully complete product research and development, including pre-clinical and clinical studies and commercialization; (ii) the Company's ability to obtain required governmental approvals; (iii) the Company's ability to attract and/or maintain manufacturing, sales, distribution and marketing partners; and (iv) the Company's ability to develop and commercialize its products before its competitors. Additional factors that would cause actual results to differ materially from those projected or suggested in any forward-looking statements are contained in the Company's filings with the Securities and Exchange Commission, including those factors discussed under the caption "Risk Factors" in the Company's Annual Report on Form 10-K/A (file No. 000-21379) filed on April 3, 2000.

Table 1: Daptomycin Phase II Clinical Summary
4 mg/kg IV Once-A-Day

	Bacteremia and RRC Combined		
Site of Infection	Bacteremia n (%)	Others n (%)	Total n (%)
Clinical Success Rate			
Modified Intent-to-Treat*	15/17 (88)	11/11 (100)	26/28 (93)
Clinically Evaluable**	12/12 (100)	5/5 (100)	17/17 (100)
Microbiologic Eradication			
Modified Intent-to-Treat*	13/17 (77)	8/11 (73)	21/28 (75)
Microbiologically Evaluable***	12/12 (100)	3/3 (100)	15/15 (93)

* Modified Intent-to-Treat: All patients with documented Gram-positive infections who receive greater than or equal to 1 dose of study medication

** Clinically Evaluable Population: Patients with documented Gram-positive infection who complete study evaluations that receive greater than or equal to 4 days study treatment and who satisfy protocol eligibility and evaluation criteria

*** Microbiologically Evaluable Population: Patients with appropriate bacteriologic cultures obtained in accordance with protocol sampling schemes (e.g. within 48 hours of initiating study therapy) and appropriate post-therapy evaluation

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CAPTION: CUBELOGO CUBIST PHARMACEUTICALS LOGO Cubist Pharmeceuticals Logo. (PRNewsFoto)[AG] CAMBRIDGE, MA USA 07/17/2000

Cubist Pharmaceuticals, Inc. Releases Online 1999 Annual Report - Company Chooses Cidecin(TM) as Trade Name for Daptomycin -

CAMBRIDGE, Mass., April 12 /PRNewswire/ -- Cubist Pharmaceuticals, Inc. (Nasdaq: CBST) today announced that its 1999 Annual Report is available electronically at the company's Web site, http://www.cubist.com. With the release of this document Cubist also announced that it has chosen the trade name Cidecin(TM) (daptomycin for injection) to describe its novel antiinfective lead drug compound.

This is the second year that the company has taken advantage of the Internet to make its annual report widely available. The company's Form 10-K statement will still be available in hard copy and were mailed to all current shareholders on or about April 10, 2000.

"We feel that the name Cidecin(TM) best conveys the potent, bactericidal nature of daptomycin," said Scott Rocklage, Ph.D., chairman, president and chief executive officer of Cubist. "Also, we are especially excited to announce Cidecin's(TM) continued development and commercialization success in our second online annual report."

Cidecin(TM) is a novel antibiotic with bactericidal activity against Gram-positive bacteria including resistant strains. It is currently in Phase III and Phase II clinical trials for the treatment of complicated skin and soft tissue and bacteremia (bloodstream) infections, respectively. Cubist also plans to initiate a global Phase III clinical trial for complicated urinary tract infection in the second quarter of 2000.

Cubist Pharmaceuticals, Inc. is a specialty pharmaceutical company focused on the research, development and commercialization of novel antimicrobial drugs to combat serious and life threatening bacterial and fungi infections. Cubist is evaluating the efficacy and safety of daptomycin in the EDGE(TM) (Evaluation of Daptomycin in Gram-positive Entities) clinical trial program.

Cubist Safe Harbor Statement

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For additional information, visit the Company's Internet web site at http://www.cubist.com or http://www.noonanrusso.com.

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Cubist and FDA Agree on Design of Additional Cidecin Phase III Clinical Trials

Company Poised to Begin Phase III Studies in Community-Acquired Pneumonia and Complicated UTI

CAMBRIDGE, Mass., July 27 /PRNewswire/ -- Cubist Pharmaceuticals, Inc. (Nasdaq: CBST) today announced progress on its Cidecin(TM) (daptomycin for injection) clinical trial program following recent meetings with the Food & Drug Administration (FDA). Cidecin is the first in a new class of bactericidal antibiotics that is being developed to treat serious and life-threatening infections, including those resistant to current therapies.

(Photo: http://www.newscom.com/cgi-bin/prnh/20000717/CUBELOGO)

Cubist announced that FDA has indicated that the design of the Phase III trials for Cidecin for the treatment of both community-acquired pneumonia and complicated urinary tract infection are acceptable. These studies will take place in the U.S. and internationally and the Company has indicated that over 150 clinical trial sites are currently being selected for participation in the trials. Pending ethics committees' approvals, Cubist anticipates that patient enrollment in these trials will begin in the fall.

Cubist also announced today that its Cidecin nonclinical package is complete and has confirmation from FDA that no additional studies will be required for registration. FDA also recently confirmed that Cubist's approach to registering the drug substance and drug product manufacturers is acceptable. Commenting on the meeting, Robert J. McCormack, Ph.D., Senior Vice President of Drug Development at Cubist said, "To date, our interactions with FDA have been very productive. We are hopeful that this cooperative atmosphere will continue and facilitate the registration process for Cidecin and any other antiinfective developed by Cubist in the future."

"It is our goal to present the most comprehensive initial NDA package possible to FDA," said Scott M. Rocklage, Ph.D., Chairman, President and CEO of Cubist. "To this end, we anticipate filing our Cidecin NDA for the indications of skin and soft tissue infection and community-acquired pneumonia, both with and without bacteremia. As additional Phase III Cidecin trials are completed," Dr. Rocklage concluded, "we will be filing supplemental NDAs for indications that may include endocarditis, complicated UTI and osteomyelitis."

A pivotal, international Phase III study on Cidecin is currently underway for the treatment of skin and soft tissue infection. To date, greater than 400 patients have been enrolled in these trials. The Company has indicated that it expects completion of patient enrollment by the end of 2000, as planned, and anticipates data and results from these trials to be released during the first half of 2001.

Cubist Pharmaceuticals is focused on becoming a global leader in the research, development and commercialization of novel antimicrobial drugs to combat serious and life-threatening bacterial and fungal infections. Cubist is evaluating the safety and efficacy of Cidecin(TM) (daptomycin for injection) in the EDGE(TM) (Evaluation of Daptomycin in Gram-positive Entities) clinical trial program and is engaged in multiple, strategic partnerships, including Novartis Pharma AG and Merck & Co. for the discovery and development of novel antiinfectives.

Cubist Safeharbor Statement

Statements contained herein that are not historical fact may be forward-looking statements within the meaning of Section 27A of the Securities Act of 1933 and Section 21E of the Securities Exchange Act of 1934, that are subject to a variety of risks and uncertainties. There are a number of important factors that could cause actual results to differ materially from those projected or suggested in any forward-looking statements made by the Company. These factors include, but are not limited to: (i) the Company's ability to successfully complete product research and development, including pre-clinical

and clinical studies and commercialization; (ii) the Company's ability to obtain required governmental approvals; (iii) the Company's ability to attract and/or maintain manufacturing, sales, distribution and marketing partners; and (iv) the Company's ability to develop and commercialize its products before its competitors. Additional factors that would cause actual results to differ materially from those projected or suggested in any forward-looking statements are contained in the Company's filings with the Securities and Exchange Commission, including those factors discussed under the caption "Risk Factors" in the Company's Annual Report on Form 10-K/A (file No. 000-21379) filed on April 3, 2000.

For additional information, visit the Company's web site at http://www.cubist.com or http://www.noonanrusso.com.

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Web site: http://www.cubist.com

Company News On-Call: http://www.prnewswire.com/comp/119304.html or fax, 800-758-5804, ext. 119304

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Cubist Pharmaceuticals Presents Phase II Daptomycin Data at the 4th Decennial International Conference on Nosocomial and Healthcare-Associated Infections

- Daptomycin, a Novel Antibiotic, Shows Promise in the Treatment of Bacteremia and Serious Gram-Positive Infections -

CAMBRIDGE, Mass., March 6 /PRNewswire/ -- Cubist Pharmaceuticals, Inc. (Nasdaq: CBST) yesterday presented positive efficacy and safety data from its ongoing dose-ranging phase II studies of daptomycin, a new class of bactericidal antibiotic being developed to treat serious and life-threatening Gram-positive infections. The dose-ranging phase II open-label clinical studies were presented at the Center For Disease Control's (CDC) 4th Decennial Conference on Nosocomial and Healthcare-Associated Infections held in Atlanta. The objective of these Phase II trials was to investigate dose selection based on clinical efficacy and safety. The combined Phase II clinical trial data showed that daptomycin, administered once-a-day at 4 mg/kg, has a 91% clinical success rate. In addition, daptomycin administered once-a-day at 4 mg/kg demonstrated a 86% clinical success rate in a subset of patients infected with a vancomycin-resistant pathogen or who were intolerant or refractory to vancomycin.

"We are very pleased with the continued clinical success of daptomycin, particularly since we've presented our findings at this important CDC conference," said Francis P. Tally, MD, executive vice president of scientific affairs at Cubist. "This data suggests that a once-a-day regimen of daptomycin is safe and effective against serious and life-threatening infections, as well as antibiotic resistant populations."

The daptomycin data discussed were from two ongoing multi-center open-label phase II studies. The first study is focused on patients diagnosed with bacteremia, a serious bloodstream infection. The second study is focused on patients who have failed or are unable to tolerate other therapies for the treatment of serious Gram-positive infections, including bacteremia, complicated skin and skin structure, complicated urinary tract infection, intra-abdominal infection and pneumonia. The combined data consisted of 63 evaluable patients in total, 56 patients were initiated on one of three doses of daptomycin (4 mg/kg once-a-day, 6 mg/kg once-a-day, or 3 mg/kg twice-a-day) and seven patients were placed on standard doses of an optimal comparator regimen, either vancomycin, oxacillin or nafcillin.

The data in Table 1, which are based on a modified intent-to-treat analysis, shows that daptomycin provided an overall clinical success rate of 91% (4 mg/kg once-a-day), 63% (6 mg/kg once-a-day) and 45% (3 mg/kg twice-a-day) of patients, compared with 71% in the comparator arm. Once-a-day dosing of daptomycin (4 mg/kg or 6 mg/kg) in bacteremic patients was clinically successful in 80% and 65% of patients, respectively. The data in Table 2 show that daptomycin had a microbiologic eradication rate of 91% (4 mg/kg once-a-day), 67% (6 mg/kg once-a-day), and 55% (3 mg/kg twice-a-day) of patients, compared with 71% in the comparator arm. The data in Table 3 show that in patients that are resistant, refractory or contraindicated for vancomycin, daptomycin had a clinical success rate of 86% at 4 mg/kg once-a-day.

Daptomycin also had a favorable safety profile similar to the comparator agents. Specifically, laboratory and clinical measures of adverse events, such as musculoskeletal, vascular or gastrointestinal, were comparable to standard treatment. Therefore, daptomycin appears to be safe and well-tolerated, with no trends in drug-related local or systemic adverse events.

"These preliminary data on daptomycin correlate well with what we have observed in patients treated with daptomycin at our medical center," said David Snydman, MD, Professor of Medicine at Tufts University Medical Center and Chief of Infectious Disease at the New England Medical Center. "Daptomycin would be an important addition to our antibiotic arsenal with the

potential to significantly aid in the treatment of patients infected with Gram-positive bacteria."

Based on these phase II data, Cubist will continue the clinical investigation of once-a-day daptomycin in patients with serious infections, including bacteremia. During 2000, Cubist plans to expand clinical studies into other serious and life-threatening infections including complicated urinary tract infections and endocarditis.

Daptomycin, a novel lipopeptide being developed by Cubist, has demonstrated potent bactericidal activity in vitro against Gram-positive bacteria including methicillin resistant staphylococci (MRSA), vancomycin resistant enterococci (VRE) and glycopeptide intermediate susceptible staphylococci (GISA). In the first quarter of 1999, Cubist commenced one US and one worldwide phase III clinical trial investigating once-a-day daptomycin (4 mg/kg once-a-day) in complicated skin and soft tissue infections.

Cubist Pharmaceuticals, Inc. is a specialty pharmaceutical company focused on the research, development and commercialization of novel antimicrobial drugs to combat serious life threatening bacterial and fungal infections. Cubist is evaluating the efficacy and safety of daptomycin in the EDGE(TM) (Evaluation of Daptomycin in Gram-positive Entities) clinical trial program. Cubist is engaged in strategic partnerships with Novartis Pharma AG, Merck & Co., Inc. and Bristol-Myers Squibb for the discovery and development of novel antiinfective products, and has formed biotechnology alliances with ArQule, Inc. and Neurogen Corporation.

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Table 1: Daptomycin Clinical Success Rates*
Modified Intent-to-Treat Population

	BAC and RRC Combined			
Site of Infection	4 mg/kg q24h n (%)	6 mg/kg q24h n (%)	3 mg/kg q12h n (%)	Comparator** n (%)
Bacteremia	8/10 (80)	11/17 (65)	5/11 (45)	5/7 (71)
All Others	11/11 (100)	4/7 (57)	N/A	N/A
Total	19/21 (91)	15/24 (63)	5/11 (45)	5/7 (71)

* Clinical Success Rates = Cure + Improvement
** BAC only

Table 2: Daptomycin Microbiological Eradication Rates
Modified Intent-to-Treat Population

BAC and RRC Combined

Site of Infection	4 mg/kg q24h n (%)	6 mg/kg q24h n (%)	3 mg/kg q12h n (%)	Comparator* n (%)
Bacteremia	8/10 (80)	11/17 (65)	6/11 (55)	5/7 (71)
All Others	11/11 (100)	5/7 (71)	N/A	N/A
Total	19/21 (91)	16/24 (67)	6/11 (55)	5/7 (71)

* BAC only

Table 3: Daptomycin Clinical Success Rates*
RRC Protocol**

Site of Infection	4 mg/kg q24h n (%)	6 mg/kg q24h n (%)	3 mg/kg q12h n (%)
All Infections	12/14 (86)	8/15 (53)	0/3 (0)

* Clinical Success Rates = Cure + Improvement
** RRC = Resistant, Refractory, Contraindicated

For additional information, visit the Company's Internet web site at http://www.cubist.com or http://www.noonanrusso.com.

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Cubist Pharmaceuticals, Inc. is focused on the discovery, development and commercialization of novel antiinfectives to treat infections caused by bacterial and fungal pathogens. Cidecin™ (daptomycin for injection), the Company's lead product, is a unique agent with potent bactericidal activity that addresses the critical need for new antibiotics to treat infections including those caused by resistant pathogens. The Company has initiated Phase III clinical trials of intravenous Cidecin for the treatment of complicated skin and soft tissue infections and a Phase II trial for bacteremia. Cubist expects to begin additional Phase III trials for Cidecin™ for the treatment of community-acquired pneumonia and complicated urinary tract infection during the second half of 2000.

Cubist has developed its proprietary VITA™ Technology (Validation In vivo of Targets and Assays for Antiinfectives) to efficiently integrate the power of genomics, proteomics, phage display technology and animal models of infection for the discovery of quality, lead compounds that inhibit validated, antiinfective targets. VITA couples the validation of antiinfective targets during an established infection in a mouse model system with assay development for the discovery of novel drug leads that bind to functionally relevant sites on targets. Cubist is applying its expertise to discover and develop novel compounds with a broad spectrum of activity against life-threatening infectious organisms such as methicillin resistant Staphyloccocus aureus (MRSA) and vancomycin resistant enterococci (VRE). In February 1999, the Company entered into a Collaborative Research and License Agreement with Novartis Pharma AG pursuant to which the Company granted Novartis a non-exclusive license to the VITA technology. In return, the Company received funds in the form of an up front equity investment, and will receive revenue in the form of research costs reimbursements, research and development milestone payments, and royalties on sales of drugs.